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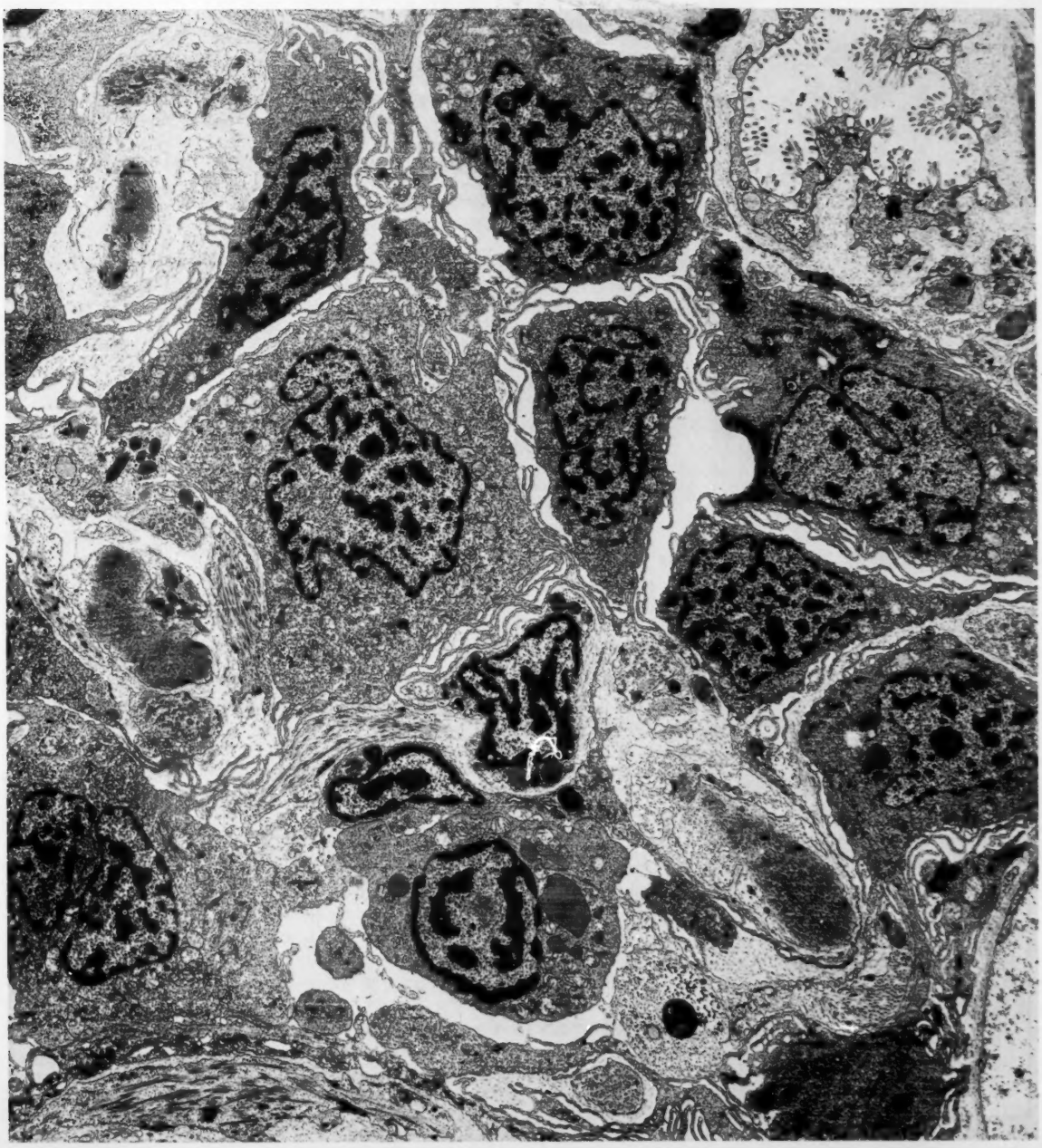

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Cover—Electron photomicrograph of *Macoma balthica* gill epithelium tissue showing an area composed primarily of neoplastic cells.

Molluscan Pathology

A workshop, co-sponsored by the Institute of Laboratory Animal Resources (ILAR), the National Research Council (NRC), and the Registry of Comparative Pathology (RCP), held 3-5 September 1975 at the Wye Institute, Wye Mills, Md.

Supported in part by funds provided ILAR through contract N-01-CM-57013 and the National Cancer Institute, National Institutes of Health, and to RCP by Public Health Service Grant RR-00301 from the Division of Research Resources, NIH, and under the auspices of Universities Associated for Research and Education in Pathology, Inc.

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Preface

This workshop was organized to examine certain salient features of the pathology of a few common species of mollusks. Those participating included marine biologists, invertebrate zoologists and pathologists, and a number of vertebrate pathologists with a special interest in the comparative aspects of disease.

Mollusks are of considerable economic importance in the fishing industry and, collectively, appear to be a major and sensitive indicator of the quality of the offshore marine environment. Thus it is important to learn more about the various proliferative lesions that are increasingly encountered in specimens taken from heavily polluted coastal waters. The key question, of course, is whether such lesions are "true" neoplasms, capable of autonomous growth, tissue invasion, metastases, and ultimate destruction of the host, or simply tissue responses to injury that are characterized by regenerative proliferation of cells.

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MFR PAPER 1201

Inflammation and Wound Repair in Oysters[1]

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When cells are injured or destroyed in vertebrates and many invertebrates, an immediate protective response is initiated. The inflammatory response is an attempt to destroy, dilute, or isolate the injurious agent and the dead or damaged cells (Sparks, 1972). The successful conclusion of the inflammatory response is wound repair. Major features of the reparative process are removal of the inflammatory exudate and restoration of the architectural integrity through regeneration or formation of scar tissue by progressive proliferation of fibroblasts and deposition of collagen.

The pioneering study in oyster inflammation was the classic study by Stauber (1950) of the role of the leukocyte in the defense mechanism of *Crassostrea virginica*. Intravascular injection of india ink results in virtual embolism in much of the arterial system followed by massive aggregation of leukocytes in and around the emboli. Within 2 to 4 hours post injection, phagocytosis is initiated. Subsequently, the ink-laden leukocytes migrate, both by passage to blood sinuses and through the walls of blocked arteries, through the Leydig tissue of the oyster and are eliminated from the oyster by migration across the epithelial borders (diapedesis), primarily of the digestive system. Diapedesis occurs rarely through the pericardial wall and through the epithelium of the mantle and palps, but almost never across the epithelia of the external shell-secreting mantle, excretory tubules, or gonoducts.

Tripp (1958) demonstrated, by intracardial injection of malarial-parasitized duck erythrocytes that intracellular digestion of digestible foreign materials occurs in addition to diapedesis. Tripp summarized the response of leukocytes to particulate matter as engulfment of the particle followed by migration. If the particle is not metabolizable, it is eliminated by diapedesis, but metabolizable materials are eliminated by a combination of intracellular digestion and diapedesis.

Pauley and Sparks (1965, 1966) studied the acute inflammatory response of the Pacific oyster (*Crassostrea gigas*) to injections of turpentine and talc. Turpentine is an irritant that elicits a typical inflammatory response in vertebrates and talc characteristically invokes a post-operative inflammation resulting in granulomatous scar tissue formation in vertebrates and has been used in a study of the wound repair process in cockroaches (Schlumberger, 1952). The use of these two substances, therefore, provided a comparison of inflammation and wound repair in vertebrates, insects, and oysters.

Grossly, injections of talc into the connective tissue result in a greenish discoloration around the wound within 16 hours which persists for 72 hours then gradually fades to a normal appearance by 96 hours. Adductor muscle injections develop an identical discoloration along the line of injection that persists, but gradually fades, through 168 hours. Turpentine injections into the connective tissue are unremarkable for approximately 32 hours at which time the adductor muscle becomes swollen and undergoes loss of function (one of the cardinal signs of vertebrate inflammation). Injection of turpentine directly into the adductor muscle elicits a similar, but earlier and more marked, gross response. The muscle becomes obviously swollen and loses its functional capability within 16 hours and frequently becomes detached from the shell after 32 hours. Abnormally large amounts of silt accumulate on the gills and mantle; pus develops in the wound within 48 hours, and grossly identifiable cellulitis is apparent by 176 hours and persists for long periods.

The first histological manifestation of the inflammatory response to talc injection is leukocytic infiltration, accompanied by edema, after about 16 hours. Blood sinuses adjacent to the injected talc become congested and contain talc after 24 hours, with congestion of large vessels some distance from the wound. Granulomatous tissue, consisting of elongate cells with round nuclei, begins forming around the talc in the larger blood vessels at about 24 hours. The nuclei of these cells gradually become elongated (by 160 hours) and the granulomatous tissue becomes well organized into thrombi that partially occlude the vessels.

Lesions begin to form in the connective tissue as early as 24 hours post injection, consisting initially of talc particles and loosely aggregated leukocytes surrounded by a compacted, peripheral band of leukocytes. The talc particles cause extensive local mechanical damage to the connective tis-

[1]Most of the material in this paper was excerpted from A. K. Sparks (1972). Reaction to injury and wound repair in invertebrates. *In* Invertebrate pathology, noncommunicable diseases. Academic Press, N.Y.

sue (Leydig) cells. After 40 hours, the peripheral leukocytes elongate and become arranged parallel to one another. Somewhat later (88 hours) leukocytes in the center of the lesion undergo karyolysis with aggregations of compact, apparently viable leukocytes present between the necrotic center of the lesion and the peripheral band of elongated leukocytes. The nuclei in the peripheral band begin to elongate between 128 and 200 hours. The central area becomes infiltrated with elongate cells by about 300 hours, and well-formed granulomas develop, consisting of a band of elongated cells 25-100 cells wide surrounding a mass of talc granules and necrotic leukocytes heavily infiltrated by elongated leukocytes. The lesions also contain large numbers of brown pigment cells after 56 hours, the function of which is unknown. Adjacent normal tissue lack such cells.

Talc injection into the adductor muscle elicits a similar response with granulomas forming in the same manner, but containing necrotic muscle fibers in the center of the lesion. Additionally, infarcts, characterized by coagulation and necrosis, are common, probably resulting from anoxia caused by partial thrombic occlusion of blood vessels.

It is clear that oysters have an effective defense mechanism against solid, nontoxic particles; they localize the particles by forming granulomas similar to those observed in insects and vertebrates. As will be shown subsequently, however, oysters do not possess the ability successfully to combat a caustic liquid such as turpentine. Such substances may spread throughout the body causing systemic necrosis of vital tissues.

Turpentine injected into the Leydig tissue causes edema and leukocytic infiltration in the area of injury within 8 hours accompanied by congestion of the adjacent smaller blood vessels and sinusoids. Marked vascular dilation develops by 16 hours with apparent leukocytic pavementing of large vessel walls in the vicinity of the injury. Increased numbers of leukocytes appear in the blood vessels and sinuses and begin to move from the blood channels towards the injury site.

By 24 hours post injection, digestive tubules and Leydig cells in the area of injection undergo massive necrosis, with the cells characterized by faded cytoplasm and pycnotic nuclei. Heavy leukocytic infiltration of the wound occurs by 40 hours and 8 hours later a conspicuous band of leukocytes surround the necrotic area. Multinucleate giant cells, normal products of postmortem change, appear at about 64 hours and are common thereafter.

A section through the injection site at this time reveals a central mass of liquified material containing fragments of destroyed cells surrounded by necrotic, but still recognizable, Leydig cells, digestive tubules, and massive numbers of infiltrated leukocytes. The architecture appears more normal and the cells more viable progressively from the center of the abcess outward. Finally a thick band (several hundred cells thick) of apparently normal leukocytes forms the periphery of the lesion. Although histologically the oyster at this point appears to have succeeded in encapsulating and thus isolating the turpentine, subsequent events demonstrate that this is not correct. Since these events are not part of the acute inflammatory response, they will not be described.

Pauley and Sparks (1967) made some rather casual observations on the repair of surface wounds caused by the needles during the injection of seawater, talc, or turpentine in their experiments on the acute inflammatory response. These led to a more detailed investigation of wound repair in *C. gigas* by DesVoigne and Sparks (1968).

Grossly, the area surrounding a surface wound (with a cataract knife) in an oyster begins to darken at approximately 16 hours after injury; this is probably analogous to the redness of vertebrate inflammation. The lesion becomes yellow-green within 24 hours and subsequently (48-96 hours) dark green. This dark coloration surrounds the wound and persists for approximately 9 days then gradually fades. However, at 28 days post wounding, when the experiment was terminated, much of the discoloration was retained.

Histologically, the first recognizable response to wounding occurs after about 4 hours. Small blood vessels in the vicinity of the lesion become pavemented by leukocytes, and infiltrating leukocytes begin to form a band underlying the mantle epithelium adjacent to the wound. Small numbers of leukocytes begin infiltrating the injured area and by 24-48 hours post injury a thick band of normal, round leukocytes surrounds the entire lesion. The blood vessels adjacent to the wound become heavily packed with leukocytes, the band of leukocytes underlying the adjacent mantle epithelium thickens, and a heavy infiltration into the region of the wound is well established.

Healing proceeds from the interior of the lesion toward the surface. Leukocytes, after delineating the margin of the wound, become fusiform and line up parallel to the wound channel. After about 160 hours the nuclei of these leukocytes also assume a fusiform appearance. As the band of fusiform leukocytes thickens around the periphery of the wound, the wound channel is heavily infiltrated by round leukocytes and fibroblasts and the wound channel is effectively plugged by 144 hours post wounding.

Subsequently the leukocytes plugging the wound channel elongate and align along the axis of the lesion. Collagen deposition becomes marked in the wound channel and normal, round leukocytes and fibroblasts continue to infiltrate along the periphery of the wound until 120-160 hours post injury. The fusiform leukocytes filling the wound channel and arranged along the axis of the lesion form randomly arranged whorls. At this point, varying in time from 88 to 488 hours post wounding, the tissue remarkably resembles a vertebrate scar. However, the original architecture is eventually restored; the whorls of leukocytes and collagen deposits are replaced by Leydig cells that are indistinguishable from normal tissue. It is not yet known whether this is accomplished by invasion of adjacent Leydig cells or by differentiation of the infiltrated, fusiform leukocytes into Leydig cells. Nor is it known how the heavy deposits of collagen are removed.

At the body surface, the band of fusiform leukocytes underlying the mantle epithelium closes the wound within 24-32 hours. The band of leukocytes gradually thickens and forms a laminated border between the underlying tissue and the exterior. The external lamina consists of fusiform leukocytes

arranged parallel to the surface; the middle lamina consists of fusiform cells perpendicular to the surface but infiltrated with round leukocytes, and the inner layer contains normal leukocytes and randomly directly fusiform leukocytes.

DesVoigne and Sparks (1968) believed that the fusiform cells in the outer lamina were totipotent, differentiating into a pseudoepithelium, then into a cuboidal epithelium and finally into a columnar ciliated epithelium identical to adjacent mantle epithelium. Ruddell (1969), however, clearly demonstrated mitotic activity and epithelial migration, beginning at 120 hours post injury and continuing for up to 720 hours. The migrating epithelial cells completely cover the wound between 144 and 200 hours after wounding. The inability of DesVoigne and Sparks to detect mitotic activity and epithelial migration despite extensive search for evidence of both these phenomena can perhaps be explained by the higher magnifications with which Ruddell worked and the incorporation of large numbers of leukocytes into the newly formed wound epithelium.

Ruddell (1969) used histochemical and electron microscopic techniques to further elucidate the wound repair process and, in addition to correcting the misconception of leukocyte differentiation into eipthelium, corroborated most of DesVoigne and Sparks' findings. He showed that copper was released in the wound by the swelling and bursting of one type of leukocyte (basophilic granular amoebocyte), that another type (agranular amoebocyte) invades the wound and phagocytizes cellular debris, and that the agranular amoebocytes differentiate into fibroblasts. Both Ruddell and DesVoigne and Sparks noted that the newly formed epithelium covering the wound is frequently hyperplastic, however, the ultimate outcome of the hyperplastic response remains unknown.

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MFR PAPER 1202

Identification of Proliferative Lesions in Mollusks

THOMAS C. CHENG

In recent years, there has been accelerated interest in the occurrence of proliferative and other types of neoplastic growth in mollusks. Consequently, although earlier summaries of reports of neoplasia in this group of invertebrates are available (Scharrer and Lochhead, 1950; Wautier, 1955; Wautier and Wautier, 1953; Pflugfelder, 1954), the number of reports has increased dramatically since the mid-1950's (see Pauley, 1969, for review). The question that needs to be asked is: Are all of the reports truly of neoplasia or are some histopathologists misinterpreting what they have observed? The reason for raising this question is because it is my opinion that some are misinterpreting leucocytic granulomas in tissue sections as proliferative neoplasms. This is an easy error to make since, morphologically, islands of immunologic cells could resemble atypical proliferating cells, even at the electron microscopical (EM) level. Specifically, at one time it was thought that finding at the EM level of elongate cytoplasmic extensions from the members of seemingly abnormal nests of cells was a reliable diagnostic feature of proliferative neoplastic cells. This idea, however, has since been discredited by Harris (1975) at this laboratory who found that certain cells contributing to the encapsulation process in the pulmonate gastropod *Biomphalaria glabrata* experimentally infected with the nematode *Angiostrongylus cantonensis* typically produce such filopodia-like projections.

Cheng

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It is recalled that the internal defense mechanisms of mollusks are primarily cellular, i.e., intra- and extravascular phagocytic hemolymph cells, which, upon recognizing the invading material as nonself, either successfully or unsuccessfully phagocytose such material. It is generally believed that if the foreign substance is dimensionally too large to be phagocytosed, then it becomes encapsulated. Although it has been suggested by Cheng and Rifkin (1970) that encapsulation may represent unsuccessful attempts of phagocytosis by many cells, experimental proof of this remains to be established. One approach to resolving this problem is to find chemical markers which would permit the identification of phagocytosis in histological sections, and see if such specific markers also occur in cells involved in encapsulation.

It must also be recalled that encapsulation may lead to the destruction and resorption of the nonself material, and when this occurs, the nonself material is no longer discernable, but an islet of the cells that comprised the original capsule remains for sometime (Cheng and Rifkin, 1968). These cells, in my opinion, comprise the type of histopathological picture that has been commonly misinterpreted as proliferative neoplastic cells. To distinguish between these reaction cells and true neoplastic cells, again, chemical markers could be useful. At the Institute for Pathobiology we have been searching for such markers, and the following is a brief review of our findings to date.

PHAGOCYTIC CELLS

Cheng and Rodrick (1975) have detected and quantified the activity levels of β-glucuronidase, acid phosphatase, alkaline phosphatase, lipase, and lysozyme, all lysosomal enzymes, in resting phagocytes of the pelecypods *Mercenaria mercenaria* and *Crassostrea virginica*. It was deemed of interest to determine whether these enzymes could be employed as markers to distinguish between phagocytes and neoplastic cells. It has since been found that lysozyme is a good marker for active phagocytes of *C. virginica* (Rodrick and Cheng, 1974), *Mya arenaria* (Cheng and Rodrick, 1974), and *M. mercenaria* (Cheng et al., 1975). Furthermore, it has also been determined that there is elevated lipase activity in active phagocytes of *M. arenaria* and the gastropod *B. glabrata* (Cheng and Yoshino, 1976 and in press), and there is a similar elevation of aminopeptidase activity in actively phagocytosing cells of *C. virginica*. It remains unknown, however, whether proliferative neoplastic cells in these species of mollusks include detectable levels of these lysosomal enzymes. If not, then cytochemical and/or biochemical identification of these hydrolases could serve to facilitate differentiation between phagocytes and pathologic cells.

ENCAPSULATION CELLS

Rifkin and Cheng (1968) have found that the encapsulation complex formed in *C. virginica* in response to parasitization by *Tylocephalum* metacestodes include glycoproteins and/or mucoproteins and neutral polysaccharides. Also, Harris and Cheng (1975) have revealed that the cellular encapsulation complex in *B. glabrata* parasitized by *Angiostrongylus cantonensis* is rich in acid phosphatase and nonspecific esterase as well as lesser amounts of alkaline phosphatase and β-glucuronidase.

Cheng (1975) has reported that the encapsulation complex in *Crassostrea gigas* formed in response to the nematode *Echinocephalus crassostreai* includes complex carbohydrates that are sulfated and rich in acidic groups, glycogen, carboxylate polyanions, and one or more of the following: neutral mucopolysaccharides, mucoproteins, and glycoproteins.

Again it is not known whether proliferative neoplastic cells include the molecules associated with cells and/or fibers involved in encapsulation in mollusks. If such do not occur, these molecules could serve as markers to distinguish between these types of cells.

RECOMMENDATION

It is being recommended strongly that the identification of neoplasms in mollusks not be based solely on gross and conventional histopathological observations since such could lead to misidentification. The use of molecular markers could serve as a useful tool in distinguishing cells involved in immunologic response and true neoplasms.

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MFR PAPER 1203

Parasitic Encapsulation in a Marine Prosobranch: The Role of Agranular Hemolymph Cells

TIMOTHY P. YOSHINO

Cerithidea californica Haldeman, a prosobranch gastropod commonly inhabiting mudflats and estuaries along the southern California coast, serves as the first intermediate host for numerous species of larval Digenea (Maxon and Pequegnat, 1949; Martin, 1955; Yoshino, 1975). As a general rule, natural infections by these parasites do not elicit cellular reactions within their host. However, an exception to this rule has been noted in infections involving sporocysts of *Renicola buchanani*, which appear to elicit a hyalinocyte-mediated encapsulation response in *C. californica*. An ultra-structural account of host capsule formation in response to this parasite is summarized in the following presentation.

HEMOLYMPH CELLS OF *C. CALIFORNICA*

From information provided by recent ultrastructural studies on encapsulation, it appears that hemolymph cells (= leucocytes or amoebocytes) represent the primary constituents involved in capsule formation in mollusks (Rifkin, Cheng, and Hohl, 1969; Cheng and Rifkin, 1970; Sminia, Borghart-Reinders, and van de Linde, 1974; Harris, 1975). Two types of cells, the granular hemolymph cell or granulocyte (Fig. 1, 3) and the agranular hemolymph cell or hyalinocyte (Fig. 2, 4) commonly are found circulating in the hemolymph of *C. californica*. Granulocytes, which measure 9.0 to 15.0 μm in diameter, possess well-defined ectoplasmic and endoplasmic regions of the cytoplasm (Fig. 1, 3), numerous vesicles of smooth endoplasmic reticulum (SER), and pleomorphic electron-dense granules (Fig. 3). Hyalinocytes, besides being smaller in size (4.0 to 8.0 μm in diameter) and in number, are distinguished from granulocytes by a general lack of SER and dense granules (Fig. 2, 4). Little or no cytoplasmic differentiation into endo- and ectoplasmic regions is noted in these cells. Finally, the cytoplasm to nucleus ratio for granulocytes (4.95 ± 1.41, $N=17$) is significantly larger ($P < 0.001$) than that for hyalinocytes (1.11 ± 0.44, $N = 16$).

CAPSULE FORMATION IN *C. CALIFORNICA*

Since experimental infections of *R. buchanani* in *C. californica* were not possible, the progression of capsule development is based on observations of host reactions to sporocysts of varying sizes found in natural infections of this snail. The smallest observed sporocysts of *R. buchanani* (approximately 0.5 mm in length) elicit little host response in the anterior mantle region of *C. californica*, though a small number of granulocytes are present in the immediate vicinity of the parasites (Fig. 5). The presence of karyolytic nuclei and cytoplasmic debris in this area suggests that cells, including granulocytes, which contact the tegument of the parasite may be subject to lytic agents of parasite origin. Granulocytes in the vicinity of young sporocysts frequently are observed phagocytizing debris from lysed host cells.

Numerous hyalinocytes begin to infiltrate the area surrounding sporocysts which measure 1.0 to 1.5 mm in length (Fig. 6). Long, thin pseudopodial processes from encapsulating hyalinocytes extend in a direction perpendicular to the sporocyst's surface, loosely interdigitating with tegumental microvilli. Heterogeneous, electron-dense inclusions commonly develop within hyalinocytes during this stage of capsule formation. Extracellular fibrils also may be present, but are not consistently observed in each capsule.

Hyalinocytes which have aggregated around mature sporocysts (2.5 to 4.0 mm in length) have become horizontally flattened against the parasite's surface forming a compact cellular tunic four to eight cell layers thick (Fig. 7). Pseudopodial processes of hyalinocytes are discernible as they interdigitate in close contact with the sporocyst's tegumental microvilli. The rough endoplasmic reticulum (RER) in these cells is usually well developed and often possesses dilated cisternae. Electron-dense inclusions and cytoplasmic microtubules also appear more numerous in capsular hyalinocytes. Sporocysts of *R. buchanani* are not harmed as a consequence of encapsulation. A normal-appearing tegumental ultrastructure and a vigorous post-encapsulation production of viable cercariae by sporocyst stages attest to this fact.

Yoshino

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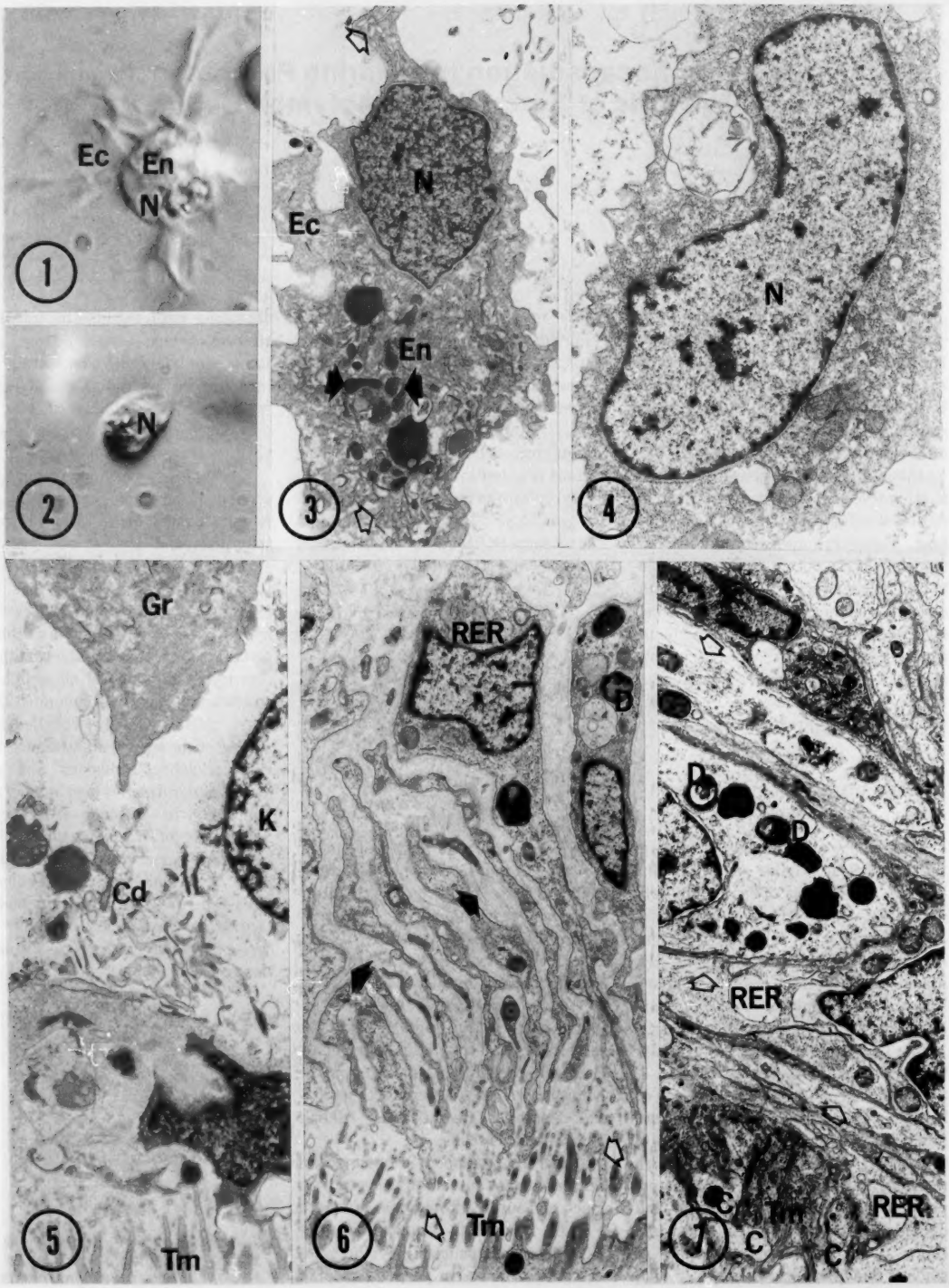
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Figure 1.—Living *C. californica* granulocyte which has been allowed to settle on a glass microscope slide. Nomarski Interference optics. 3,000×. Figure 2.—Living *C. californica* hyalinocyte which has been allowed to settle on a glass slide. Nomarski Interference optics. 3,000×. Figure 3.—Granulocyte in hemolymph circulation of *C. californica*. Note the presence of numerous vesicles of SER (hollow arrows) and electron-dense granules (solid arrows). 8,800×. Figure 4.—Hyalinocyte in hemolymph circulation of *C. californica*. Note the general lack of SER and dense granules. 15,000×. Figure 5.—Pre-encapsulation encounter between host granulocyte (Gr) and 0.5 mm *R. buchanani* sporocyst. Note the karyolytic nucleus (K) and scattered cytoplasmic debris (Cd) from lysed host cell in the immediate vicinity of the sporocyst's tegument (Tm). 10,000×. Figure 6.—Hyalinocytes surrounding *R. buchanani* sporocyst (1.0-1.5 mm in length) at an early stage in capsule formation. Thin cytoplasmic processes from hyalinocytes (hollow arrows) loosely interdigitate with sporocyst microvilli (Tm). Extracellular fibrils are indicated by solid arrows. 8,000×. Figure 7.—Layers of flattened hyalinocytes surrounding a 4.0 mm *R. buchanani* sporocyst. Cytoplasmic processes (C) are intimately bound up with tegumental microvilli (Tm). Cytoplasmic microtubules in hyalinocytes are indicated by hollow arrows. 9,000×.

CONCLUDING REMARKS

Of the two types of hemolymph cells found in *C. californica*, only the hyalinocyte plays an active role in the encapsulation of *R. buchanani* sporocysts. However, whether these cells merely serve as a physical barrier between host and parasite, or whether they actually represent a physiological or biochemical barrier is at present undetermined. The strong development of RER and dense inclusions in capsular hyalinocytes suggests that these cells may be undergoing functional changes, and supports the supposition that hyalinocytes are providing more than a spacial separation between host and parasite tissues. As has been pointed out by Cheng (1976) at this workshop, the ability of cells to react with and eliminate foreign materials may be related to the compatibility of enzymes elaborated by hemolymph cells and the foreign substrates. Certainly the basic biochemical mechanisms which allow for the establishment of a benign relationship between encapsulating hyalinocytes and *R. buchanani* sporocysts are in need of further investigation.

ACKNOWLEDGMENTS

This research was supported in part by National Science Foundation Grant No. GA 34144 to E. R. Noble (University of California, Santa Barbara) and in part by U.S. Public Health Service Research Fellowship No. AI05194-01.

ABBREVIATIONS USED IN FIGURES

C	cytoplasmic processes
Cd	cytoplasmic debris
D	dense inclusions
Ec	ectoplasmic region
En	endoplasmic region
Gr	granulocyte
K	karyolytic nucleus
N	nucleus
RER	rough endoplasmic reticulum
Tm	tegumental microvilli

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MFR PAPER 1204

Effects of Phenol on Clams

C. FRIES and M. R. TRIPP

Adult hard clams (*Mercenaria mercenaria*) were collected from Rehoboth Bay, Del., in October 1974 and April 1975. Within less than 24 hours they were divided into groups of six; control animals were placed in artificial seawater (25‰) while experimental animals were placed in phenol solutions in artificial seawater (25‰) with initial concentrations of phenol of 1, 5, 10, 25, or 50 ppm. After 24 hours clams were placed in Bouin's fixative and 4 hours later three transverse sections 0.5-0.7 cm thick were cut from each clam at the level of digestive gland, gonad, and heart. These sections were fixed in Bouin's for an additional 24 hours, then washed, dehydrated in alcohol, cleared in xylol, embedded in Paraplast[1], sectioned at 10 μm, and stained with Harris' alum, hemotoxylin, and eosin.

Microscopic examination of tissues of control animals revealed that epithelia were intact and free of significant cell damage. Gill epithelium consisted of tall columnar ciliated epithelial cells well-stained and cohesive (Fig. 1). Epithelium of intestine was intact, well-stained and normal in all respects. Digestive diverticular tubules were intact, staining darkly in the basal areas and lightly vacuolated in the distal portions of the epithelial cells. Other tissues (mantle, foot, gonad, heart, kidney) appeared normal and undamaged.

Gills of clams treated with 1 or 5 ppm phenol showed moderate to extensive epithelial necrosis (Fig. 2); hemolymph sinuses were distended and contained an amorphous precipitate (presumably denatured hemolymph protein). Exposure to greater concentrations (10, 25, and 50 ppm) of phenol resulted in greater gill damage. Epithelial necrosis and sloughing was massive, and at 50 ppm the only recognizable gill structure remaining was the chitinous rods (Fig. 3).

Gut epithelia of clams exposed to 1 ppm (or higher) phenol also showed extensive necrosis and sloughing (Fig. 4). The regular palisade structure of normal tissue was disorganized, ciliation was irregular, and patches of epithelium became detached. These effects became more pronounced with increasing concentrations of phenol. Digestive diverticula were affected similarly; extensive sloughing lead to massive disorganization of the digestive tissue (Fig. 5). Initially damage to tubule epithelia might be localized, but in some animals at high concentrations, the damage involved the whole digestive gland. Damage to other tissues was less spectacular and more irregular.

Posterior portions of hind gut epithelium did not seem quite as sensitive as esophagus or stomach, but at 10 or 25 ppm phenol necrosis and sloughing was detected. Mantle connective tissue occasionally was disorganized and distended blood vessels contained precipitated hemolymph, but epithelium seemed little affected. Foot epithelium and muscle seemed unaffected at any phenol concentration tested. Sections through the kidney were not always obtained and it is not clear if that organ was affected. Gonadal tissue was not affected.

It is clear that gill and digestive tract epithelia are damaged by phenol at concentrations of 1 ppm or greater for 24 hours or longer. Experiments are in progress to measure the ability of clams to heal these chemical wounds and to detect subcellular damage at lesser concentrations. The extensive damage to gill and gut tissues thought to be important components of intrinsic defense mechanisms, suggests that such phenol-treated clams may be more susceptible to microbial infection and disease than normal ones. This system may serve as a model for studying host-parasite-environment interactions.

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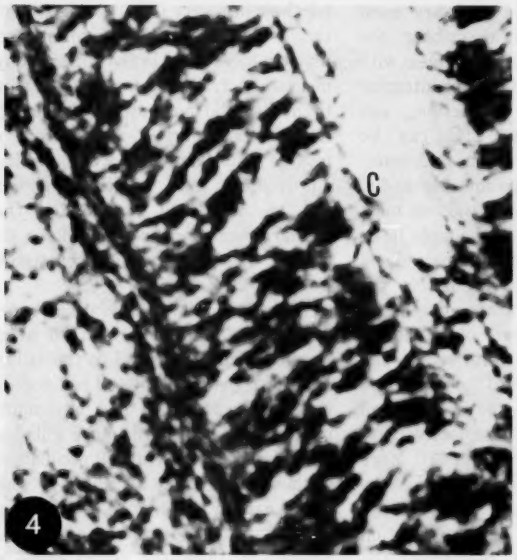
[1] Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.



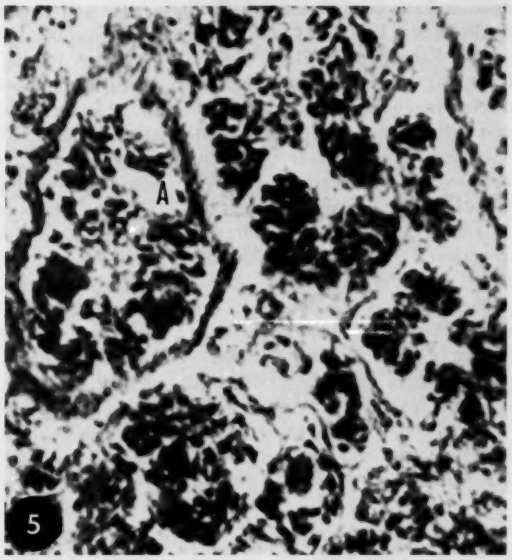


Figure 1.—Normal gill. Uniform ciliated columnar epithelium and highly vascularized underlying connective tissue. 20×. Figure 2.—Gill of clam treated with 5 ppm phenol for 24 hours. Note lack of epithelial cells and disorganized underlying connective tissue. 20×. Figure 3.—Gill of clam treated with 50 ppm phenol for 24 hours. The filaments have been stripped of their epithelium and only the chitinous supporting rods remain. Underlying connective tissue is necrotic. 20×. Figure 4.—Gut epithelium of clam treated with 1 ppm phenol for 24 hours. The epithelial cells are necrotic and not cohesive; ciliation (c) is reduced. 200×. Figure 5.—Digestive diverticulum of clam treated with 25 ppm phenol for 24 hours. Complete disorganization of absorptive cells is evident (a). Connective tissue is necrotic. 200×.

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MFR PAPER 1205

A Review of the Histopathological Effects of Ionizing Radiation on the Pacific Oyster, *Crassostrea gigas*

MICHAEL C. MIX

It has long been known that ionizing radiation causes deleterious effects in mammalian cells and tissues (Bergonie and Tribondeau, 1906). During the past 30 years, the mechanisms responsible for the various mammalian radiation syndromes of cellular and tissue degeneration have been elucidated (Bacq and Alexander, 1961; Patt and Quastler, 1963; Fabrikant, 1972).

Excepting studies of the effects on planarian regeneration, and those associated with "sterile male" techniques for biological control of insects, there have been few studies concerned with the pathological effects of ionizing radiation on metazoan invertebrates. During the early 1970's, we published a series of papers on the histopathological effects of ionizing radiation on the Pacific oyster, *Crassostrea gigas* (Mix and Sparks, 1970; Mix and Sparks, 1971a, b; Mix, 1972). Unfortunately, at that time little was known about normal cell renewal systems (CRS) of bivalve mollusks and thus, we were forced to interpret many of the results of those studies without adequate knowledge of these systems. Since that time, my graduate students and I have corroborated some of the assumptions and tentative descriptions of bivalve CRS made during those studies.

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Radiation effects at the tissue, organ, and organism level are most easily explained by describing the effects on normal CRS. Tissues with cell turnover depend for their integrity on continued cell production, and perturbations of the CRS can be brought about, perhaps most dramatically, by exposure to ionizing radiation. The effects of irradiation on most CRS can be reduced to a simple basic scheme; initially, there is impaired cell production with little change in the rate of cell loss, which leads to cell depletion; the degree of depletion depends on the extent to which cell production is impaired. Thus, radiation causes aplastic cytopenia or, if a cell population vanishes completely, acytosis (Patt and Quastler, 1963). More or less complete restoration of the system may be possible if the organism survives until stem cell proliferation can be successfully resumed. The effects of irradiating certain oyster tissues and cells can be explained within this scheme, although it is difficult to make precise analyses because of the paucity of knowledge about cell kinetics in these animals.

Basically, oysters were irradiated with various doses of gamma radiation (0, 1, 10, 20, 50, 100, 200, and 400 krads), maintained in a continuous flow-through seawater system 9°-15°C (ambient temperatures) and sampled for 90 days during the first study and 180 days during a second study in which all oysters were irradiated with 75 krads. All oysters were prepared for histological examination and over 1,000 animals were analyzed during these studies (see Mix and Sparks, 1970; Mix and Sparks, 1971a, b; and Mix, 1972 for additional details).

A brief summary of the most significant results is included below.

1) All oysters irradiated with 200 and 400 krads died within 9 days while oysters receiving 0-100 krads did not experience mortality within the 90-day experimental period (Mix and Sparks, 1970).

2) Oysters irradiated with 75 krads died approximately 180 days post-irradiation (Mix, 1972).

3) Chronic degeneration, thought to be caused by mitotic inhibition and/or complete failure of cellular or subcellular repair mechanisms, was evident in all tissues dependent on CRS for their integrity (Fig. 1A-H). Quantitative data indicated that mitotic cell division had ceased in the gut by 8 days in oysters irradiated with 10 or more krads and did not resume during the experimental period. As a result of mitotic inhibition, there was a continuous decline in the epithelial cell population of this tissue. It was felt that similar phenomena accounted for the chronic degeneration of other tissues (Mix, 1972).

4) The mortality that occurred by 180 days after irradiation with 75 krads was presumably related to the failure of CRS to restore depleted cell populations and was thought to be particularly important with regard to the gills and digestive tubules. The cell populations in these two tissues by 180 days were either greatly diminished (gills) or had completely disappeared (digestive tubules) and thus, the actual cause of death was probably starvation.

5) Restoration of injured and destroyed cell populations was observed in only one tissue, the digestive tubules, in oysters irradiated with sublethal doses. Desquamated tubules were repopulated after rapid proliferation of unusually large crypt cells (Mix and Sparks, 1971a). It is interesting to note that these cells did not differentiate; it is not known if this was due to low water temperatures or perhaps the absence of a "maturing factor." There

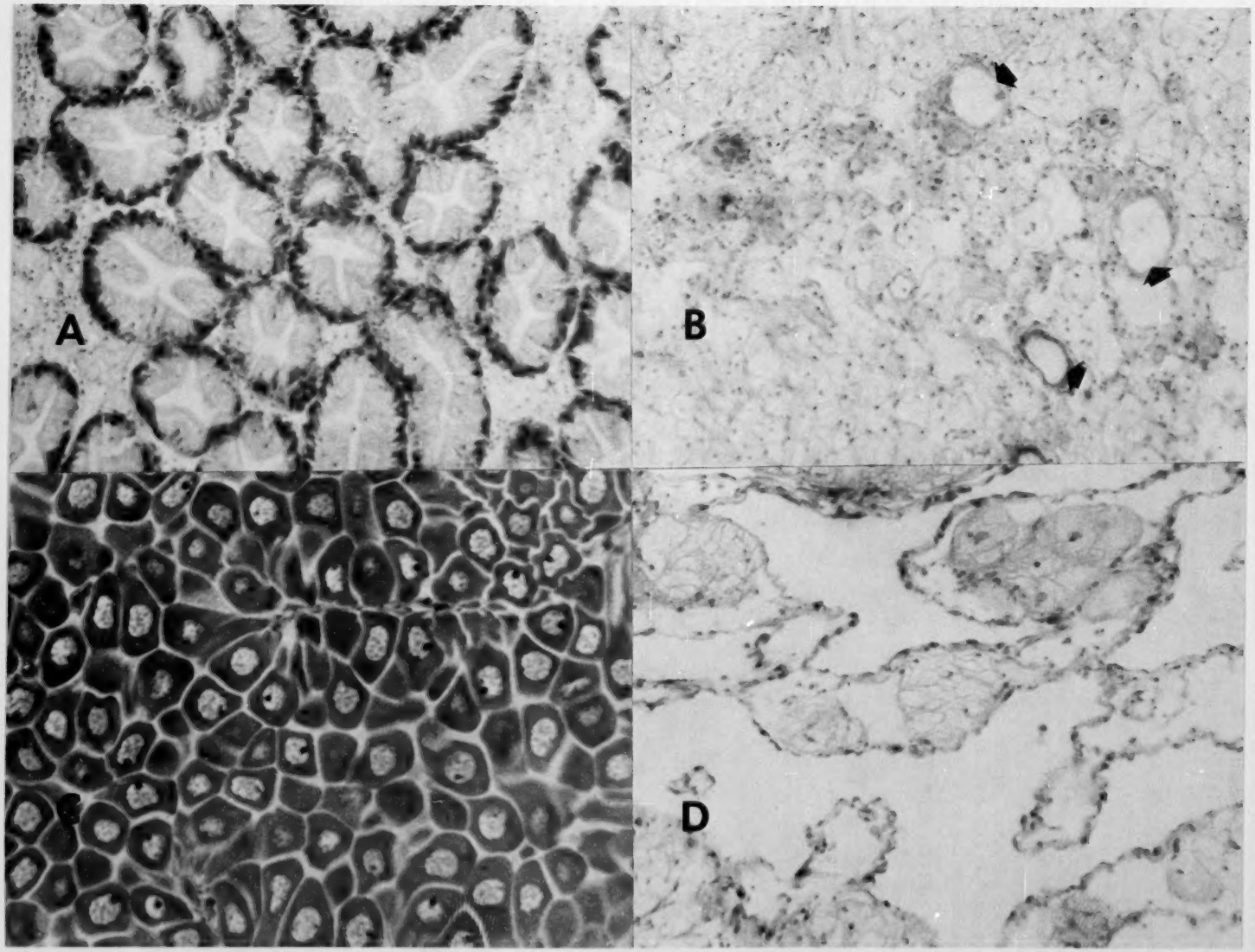

Figure 1 (A-D).—Normal tissues of *Crassostrea gigas* 180 days after sham irradiation and tissues 180 days after irradiation with 75 krads (128×). A. Normal digestive tubules. B. Digestive tubule area after irradiation; arrows indicate denuded tubules which were not repopulated. C. Normal gonad containing mature ova. D. Irradiated gonad. All oysters were maintained in the same system.

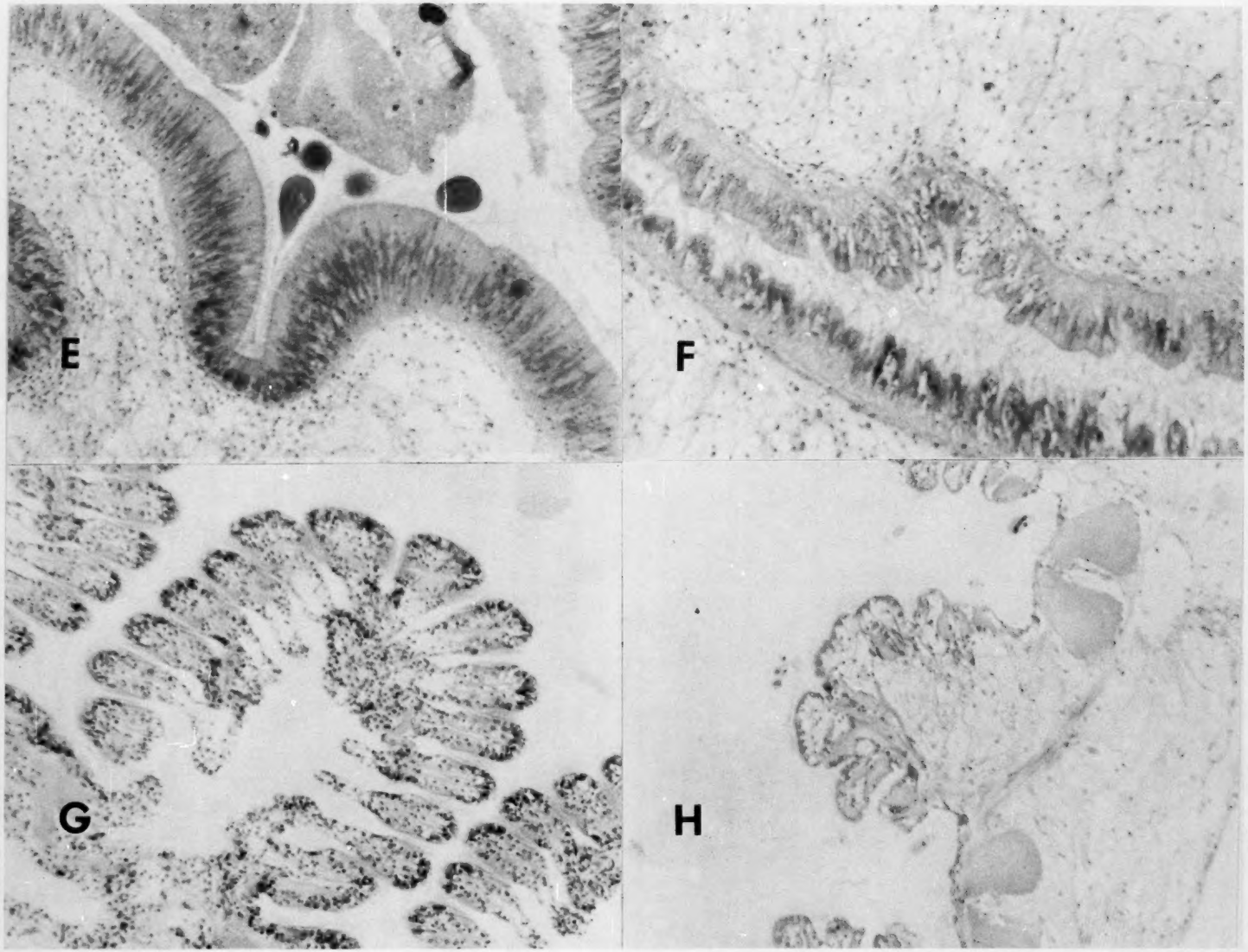

Figure 1 (E-H).—Normal tissues of *Crassostrea gigas* 180 days after sham irradiation and tissues 180 days after irradiation with 75 krads (128×). E. Normal gut containing unassimilated food. F. Irradiated gut. G. Normal gill. H. Irradiated gill. All oysters were maintained in the same system.

was no evidence to indicate that tubules repopulated by the undifferentiated crypt cells ever became functional.

6) Doses as low as one krad caused degeneration of the gonad in some oysters and doses in excess of one krad effectively sterilized all oysters. It appeared that sterilization was permanent as evidenced by the formation of scar-like tissue in the gonad (Mix and Sparks, 1971b).

The effects of ionizing radiation on *C. gigas* can be explained and interpreted, in part, by analyzing the effects on normal CRS. The evidence suggests that depletion of essential cell populations was directly or indirectly responsible for death of the oyster which occurred 5-6 months after irradiation with 75 krads.

From our studies, certain conclusions can be made about tissue sensitivity and restoration of depopulated tissues in *C. gigas*. Since cell depletion rates are directly related to cell turnover times, the order of radiosensitivity in oyster tissues is probably (most to least sensitive): Gonad, gill, digestive tubules, mantle, and other digestive tissues. Leydig, muscle, and nerve cells are quite resistant, which is consistent with their apparent inability to proliferate. *Crassostrea gigas* gills appear to possess certain cellular mechanisms, first described in the mammalian gut, which can partially compensate for the accelerated rate of cell loss caused by ionizing radiation; these include a delay in the rate of cell loss by extension of the transient time spent on the gill filament and hypertrophy of remaining cells which prevents denudation of the filament. Digestive tubule cells may have exhibited a mechanism associated with accelerated cell repopulation in some mammalian tissues —inhibition of differentiation until the cell population of a tissue is restored.

It seems apparent that the LD_{50} concept so widely used in radiation biology has little practical value in studies on metazoan invertebrates. It is evident that because of slow cell turnover times, radiation effects may not manifest themselves until months or even years after irradiation. The old idea that invertebrates are resistant to radiation can no longer be accepted unequivocally. Careful studies utilizing low doses of radiation and extended time periods are clearly necessary to establish the exact sensitivity of any particular invertebrate.

There are many unanswered questions which will hopefully be the subject of future investigations. Among them are these:

1) Do bivalve mollusks possess subcellular repair mechanisms or were the digestive tubule cells involved in repopulation simply survivors and not "repaired cells?"

2) What are the kinetics of tissue repair?

3) What are the effects of temperature on subcellular repair and cell and tissue renewal?

4) How does temperature affect the rate of tissue degeneration and subsequent death of the organism?

5) Why did the digestive tubule cells involved in tissue repopulation fail to differentiate?

6) What doses can cause more subtle negative biological effects (mutations, neoplasia, life-span shortening)?

7) How do leucocytes fit in; are they involved in tissue repair; what is the effect of irradiation on leucocyte cell renewal?

Finally, it is obvious that in order to answer many of these questions it will be necessary to utilize autoradiographic techniques. Unfortunately, attempts to apply such methods on *C. gigas* have failed (Mix, 1972; Mix and Tomasovic, 1973) and thus, it may be necessary to select a different species for use as a model in understanding the effects of ionizing radiation on bivalve mollusks.

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MFR PAPER 1206

A Proteomyxan Amoeba Stage in the Development of *Labyrinthomyxa patuxent* (Hogue) Mackin and Schlicht, With Remarks on the Relation of the Proteomyxids to the Neoplastic Diseases of Oysters and Clams

J. G. MACKIN and FRANK G. SCHLICHT

Hogue (1921) described *Vahlkampfia patuxent*, an amoeboid protist parasite of oysters (*Crassostrea virginica*) from Chesapeake Bay. Studies of these parasites have shown that early stages of development involve a proteomyxan amoeba, which, under certain environmental conditions, causes lethal epizootic disease in host populations. This report is intended to describe these amoebae. It is believed that all serious students of molluscan pathology have observed these protistans, but have failed to recognize their significance. This report will be concerned only with the early amoeboid stages of the development of *Labyrinthomyxa patuxent*. Later development follows closely the steps described for *Labyrinthomyxa marina*, for which see Mackin (1962). *Labyrinthomyxa marina* passes through all the developmental stages here described for *L. patuxent* including the amoebae, but differs quantitatively, as indicated below.

Earliest uninucleate amoebae, as removed from the haemolymph of oysters in advanced disease, are rounded to oval, about 6 to 12 μm in length, and may have one or two polar pseudopods (Fig. 1), which are curved, lamellate, and capable of a characteristic lateral movement. Growth and further development of these cells results in: 1) Production of endogenous buds (Fig. 2a, b) ranging in number from one to many depending on the size of the amoebae; 2) growth to giant amoebae (Fig. 3a, b, c) which may be more than 200 μm in length when extended; and 3) production of multibranched and anastomosing pseudopodia (Fig. 4) originating from flattened ectoplasmic lamellae. Endogenous buds may produce secondary gemmae (Fig. 2b), and rarely even more. Endogenous buds are extruded from the amoebae, leaving a rounded cavity, which may persist for a period (Figs. 5, 10) in the mother cell. Early buds are rounded uninucleate cells which resemble oyster leucocytes when in stained sections (Fig. 6) and which can reproduce by binary fission to produce massive concentrations in the host tissues and haemolymph (Fig. 7). Later endogenous buds may form small plasmodia, sporangia, and presporangia, often in the same amoeba (Fig. 8). *Labyrinthomyxa patuxent* differs from *L. marina:* 1) In its incomplete response to Ray's (1954) thioglycollate diagnostic culture method, apparently not responding in early ameoboid stages, but responding positively only to presporangia or sporangia; 2) in the relatively greater prominence of early amoeboid stages; 3) presporangia are on the average slightly smaller than the same stage in *L. marina*; and 4) spindle cells of *L. patuxent* are spindle shaped when grown in culture, while those of *L. marina* are round to oval. Both contain a large lipid granule in an eccentric vacuole, and an eccentrically placed nucleus.

Labyrinthomyxa patuxent has the characteristics of the theoretical neoplasms of oysters and clams described by Couch (1969), Farley (1969a, b), Christensen, Farley, and Kern (1974), and others. Sparks (1972) summarized these as: 1) Nuclear enlargement; 2) an unusual number of mitoses in stained preparations, of which some appear to be multipolar; 3) an excess of chromosome number over those in host cells;

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Figure 1.—Two uninucleate cells with polar flattened pseudopods, which can be moved laterally.

Figure 2a.—Early development of endogenous buds in a small proteomyxan amoeba. Figure 2b.—A later stage, with several endogenous buds, one of which has produced a secondary bud of its own.

Figure 3a.—A giant proteomyxan amoeba, contracted into a spherical mass, but with short pseudopods around the periphery. This amoeba contains a large number of endogenous buds in the center. Figure 3b.—Same as Figure 3a, but partially extended, showing the clear ectoplasmic lamellae. Figure 3c.—A fully extended amoeba, except for the pseudopods, photographed at 450 diameters. This amoeba measured about 150 μm in length.

Figure 4.—A small amoeba with fully extended pseudopods, which measure about 75 μm. The pseudopods are branched and subbranched, and arise from one of the extended lamellar ectoplasmic projections.

Figure 5.—A proteomyxan amoeba showing two large vacuole-like cavities, which were occupied by buds which have escaped.

Figure 6.—A proteomyxan amoeba in stained section of the host oyster, *C. virginica*. Here the thin layer of cytoplasm is hardly noticeable around the endogenous buds.

Figure 7.—Stained section taken at 450 diameters to show the large number of amoebae in a heavily infected oyster.

Figure 8.—A proteomyxan amoeba in late stages of disease. The rounded vegetative cells are now replaced as endogenous buds by presporangia (left side of amoeba section), small sporangia with tightly appressed nuclei (right of section), and only one vegetative cell.

Figure 9.—A live cyst attached to gill tissue (note the double wall).

Figure 10.—Several large myxamoebae with vegetative cells, most of which are ready to leave the mother cell, and show the vacuoles in stained preparation, which are shown in vivo in Figure 5.

Figure 11.—An enlarged nucleus of *Mytilus edulis*, from Yaquina Bay, Oreg., stained by C. A. Farley. This nucleus is in process of dividing after an intra-nuclear mitosis. The median plate is clearly shown. (Courtesy of C. A. Farley.)

All photomicrographs were taken at 1,125 diameters, except for Figures 3c and 7. All stained sections were stained with Wolbach's modification of the tissue giemsa. All in vivo photomicrographs were taken using amoebae removed from the hemolymph of oyster number 6-25-75+2 (*Crassostrea virginica*) from West Bay, Tex.

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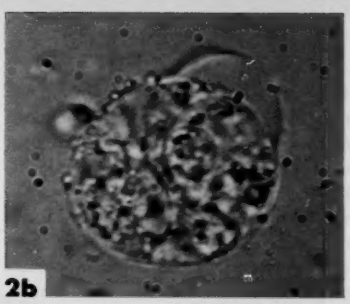
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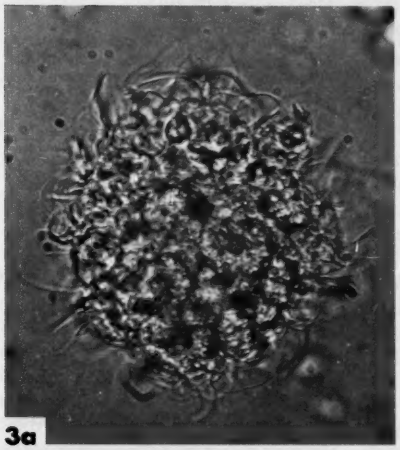
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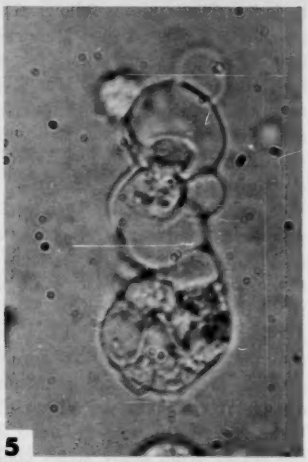
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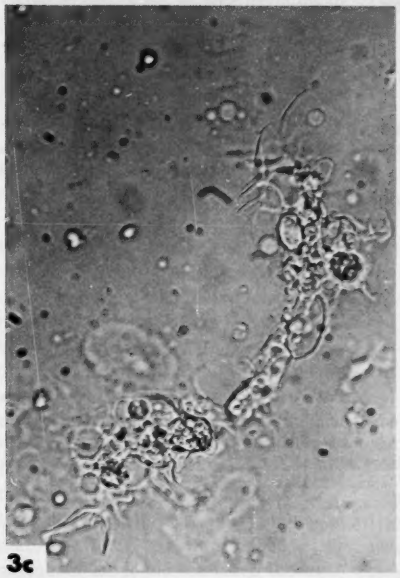
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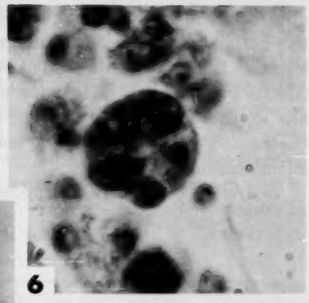
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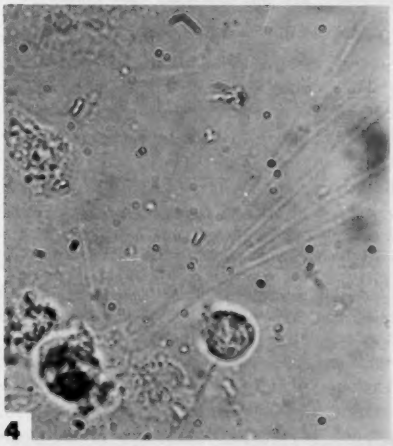
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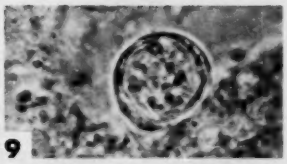
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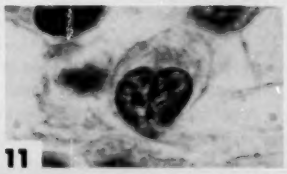
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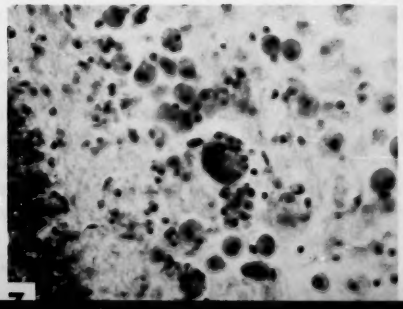
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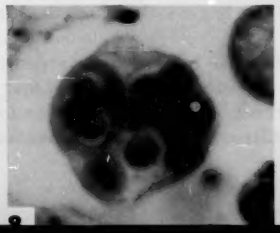
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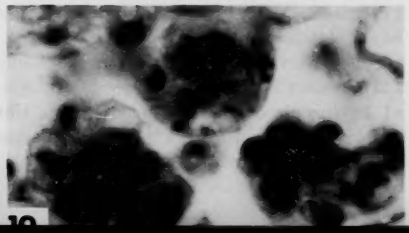
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4) frequent lobed nuclei; 5) a prominent nucleolus, and sometimes two or more of these in a single nucleus; and 6) a sparse but highly basophilic cytoplasm, bi- and multinucleate cells. It was assumed that no known protist had these characteristics (Sparks, 1972). The cells, assumed to be of haemopoietic origin, also have certain other characteristics. These are as follows: 1) The mitoses are intranuclear, a characteristic of protistans; 2) the cells frequently become encysted (Fig. 9); 3) they may be formed as endogenous buds; and 4) in addition to "normal" mitoses, some of the mitotic figures are of the cruciform type characteristic of the Plasmodiophorales (see Karling, 1944) and the problematic *Phagomyxa algarum* (Karling, 1944), which the author believes is a species of *Labyrinthula* Cienkowski (1867).

Only one group of the protista combines all of the characteristics described for the assumed neoplastic diseases by the various authors. The list of characteristics was added to by the author from study slides contributed for use of participants at the shellfish pathology conference. This group is the Labyrinthulales Cienkowski (1867). Plasmodiophorales apparently has all characteristics excepting only the capacity to reproduce by endogenous budding.

Analysis of the papers of proponents of the neoplasm theory shows that their case is based on the fallacious assumption that mitoses observed in the enlarged cells are abnormal. Particularly singled out is the apparent presence of polycentric mitoses. These are, in fact, not polycentric. In the enlarged nuclei, the product of the first mitosis is two nuclei, both occupying the area of the original one nucleus, and flattened against each other (Fig. 11). On initiation of the second division in the two daughter nuclei, division of the nucleoli precedes the formation of the equatorial plate. A peculiarity of the group is that the poles thus established by the nucleoli may lie at right angles to each other in the two daughter nuclei. In this manner simultaneous mitoses in the two daughter nuclei may be interpreted as one polycentric mitosis. Because the tetrad of small nuclei, still confined to the area of the original large nucleus, may initiate further crowded mitoses, the confusion is compounded.

The burden of proof lies with the proponents of the "neoplasm" theory. They must prove that carcinogenic or radiational damage to leucocytes of shellfish, or spontaneous changes in leucocytes, can produce alteration of chromosome number ending in a larger number by a significant margin. They must also prove that leucocyte mitoses are intranuclear, that leucocytes may encyst, and that they may be multinucleate.

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MFR PAPER 1207

Dermocystidium marinum Infection in Oysters

Perkins

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A review of knowledge concerning the life cycle, structure, taxonomy, and pathology of *Dermocystidium marinum* is presented. The pathogen causes mortalities of the American oyster, *Crassostrea virginica*, along the Atlantic and Gulf coasts of the United States. The life cycle consists of vegetative reproduction in which uninucleate aplanospores enlarge and undergo successive bipartition (alternating karyokinesis and cytokinesis) to form 4- to 64-cell sporangia from which are liberated uninucleate, coccoid or cuneiform aplanospores which are 2-4 μm in longest axis. Ultrastructure of recently liberated aplanospores includes a pair of centrioles resting in invaginations of the nuclear envelope. The organelles contain a large cylindrical, electron-dense granule in the lumen and have a nine-fold, triplet blade microtubular substructure typical of many eucaryotic cells. A granular wall is continuous around the cell often having host cell phagosome membranes embedded in the wall. One to three lomasomes are found beneath the wall in random locations at the cell surface. Tubulovesicular mitochondria with electron-dense material filling the interior of many segments are found scattered throughout the cytoplasm (Perkins, 1969).

As aplanospores enlarge and mature, a large eccentric vacuole develops which may occupy greater than 50 percent of the total cell volume. At this stage the cells are about 5-10 μm in diameter. From the vacuole membrane is synthesized electron-dense material which accumulates in the vacuole and forms a free-floating refringent vacuoplast (Fig. 1).

Cytokinesis occurs by plasmalemma invagination after each nuclear division. Daughter cells initiate wall synthesis after the full complement of cells has been reached and before the mother cell wall has ruptured. After rupture, mother cell wall fragments may adhere to the aplanospore.

In moribund oysters uninucleate prezoosporangia may be rarely formed by marked enlargement of aplanospores to about 15-20 μm. The mechanism appears identical to that which occurs in fluid thioglycollate medium (Ray, 1952; Perkins and Menzel, 1966), except that the medium induces enlargement to sizes above 20 μm, cells being observed larger than 100 μm in diameter. Enlargement results in cells with a very large eccentric vacuole which may comprise 90 percent of the cell volume.

Liberation of prezoosporangia from oyster tissue into seawater results in zoospore formation by successive bipartition of the protoplast and subsequent release from the zoosporangia. During zoosporogenesis the large vacuole subdivides as the daughter cells progressively decrease in size. Zoospores initiate infections by establishing themselves or being established by amoebocytes in epithelia. Zoospores then become aplanospores and multiply as described above.

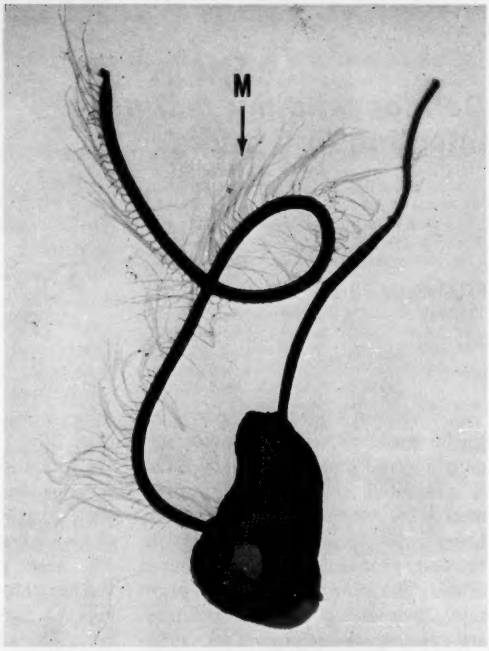
Ultrastructural studies of various cell types in the life cycle reveal numerous characteristics of the Coccidia (Scholtyseck, 1973). *Dermocystidium marinum* zoospores have a conoid at the anterior end of the cell with several rhoptries terminating in the lumen (Fig. 2). Similar membrane-bound sacs containing electron-dense material, but which do not terminate in the conoid lumen, are found in the anterior half of the cell. Those organelles may be analogous to micronemes. A cytoskeleton of microtubules radiate from a polar ring around the anterior end of the conoid and pass posteriorly through most of the cell length just beneath the plasmalemma (Fig. 2). Micropores have not been found in motile zoospores, but are found in all vegetative cell stages not engaged in zoosporogenesis (Perkins, 1969). The organelle resembles those found in *Eimeria* spp. (Scholtyseck, 1973).

Dermocystidium marinum zoospores are biflagellate as are the microgametes of several Coccidia, but appear to differ in that mastigonemes are present (Fig. 3). Although coccidian microgametes appear to lack mastigonemes, this point has not been adequately studied using the necessary preparations of negatively stained or shadowed whole mounts. There is no evidence that *D. marinum* zoospores are microgametes.

The numerous structures (conoids, polar rings, an ordered cytoskeleton of microtubules beneath the plasmalemma, rhoptries, micronemes, and micropores) found in *D. marinum* and considered by protozoologists to be characteristic of the Coccidia lead me to suggest that the oyster pathogen is a coccidian or at least closely related. In my studies I have found no evidence

Figure 1.—Developing aplanospore of *Dermocystidium marinum*. Eccentric vacuole with vacuoplast (V); lomasome (L); and nucleus (N). 14,000×.

Figure 3.—Whole amount of *D. marinum* zoospore. Mastigonemes (M) along one side of anterior flagellum. 11,000×.



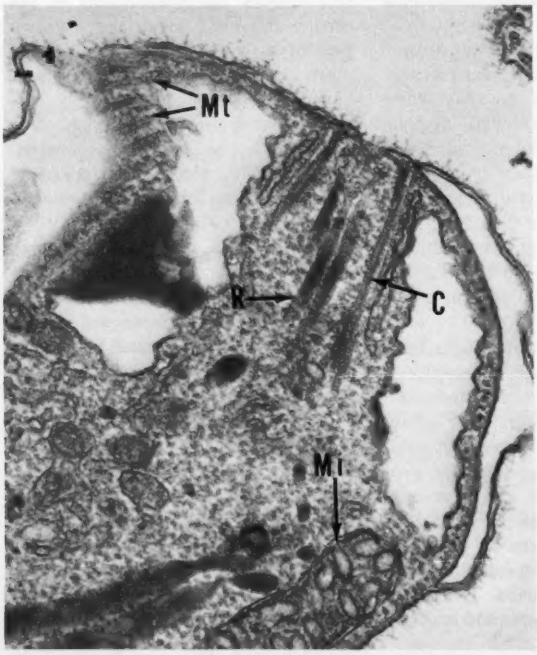


Figure 2.—Anterior end of zoospore showing conoid (C), microtubules (Mt) of cytoskeleton, mitochondrion (Mi), and rhoptries (R) within conoid. 64,000×.

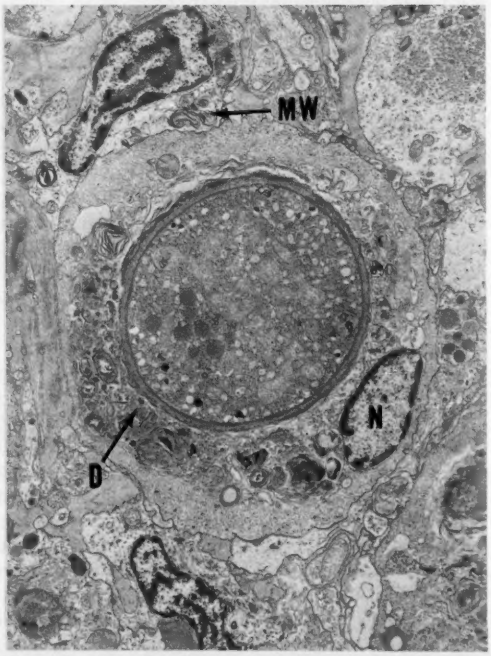


Figure 4.—Aplanospore in oyster amoebocyte of mantle connective tissue showing degenerative changes in host cell. Amoebocyte nucleus (N) is pycnotic and cell debris (D) is found around the aplanospore. Connective tissue and muscle cells of mantle appear less severely affected; however, myelin whorls (MW) are present in cells nearest the amoebocyte. 9,000×.

that *D. marinum* is a labyrinthulid (see Olive, 1975, for review of the group) as suggested by Mackin and Ray (1966), the assumption being made that *Labyrinthomyxa* spp. are labyrinthulids. The oyster pathogen lacks labyrinthulid characteristics such as a bilateral row of mastigonemes on the anterior flagellum and formation of ectoplasmic nets from sagenogenetosomes (Perkins, 1972). Labyrinthulids lack the coccidian characteristics mentioned above. One unusual ultrastructure which *D. marinum* and labyrinthulids have in common is the large cylindrical kinetosome and centriole granule; however, such an inclusion is also found in ciliates (Allen, 1969; Didier, Iftode, and Versavel, 1970).

The histopathology of *D. marinum* infections is essentially as already described by Mackin (1951). Any cell stage appears capable of initiating infections except possibly prezoosporangia. Infections are initiated in the gill, mantle, or gut epithelia or in the connective tissue near the basement membrane. Cells of the pathogen may lie between or within host cells (Fig. 4). Multiplication results in a cluster of uninucleate aplanospores which spread through the host via amoebocytes. Extensive leucocytosis, noticeable at the tissue level, may or may not occur.

Foci of pathogen cells are established in the connective tissue and epithelia in which multiplication proceeds as described above resulting in progressive degeneration among juxtaposed host cells. Tissue response in the early stages often consists of an attempt at "encapsulation" by several layers of host amoebocytes; however, as the pathogenic cells increase in number the "capsule" disappears. In later stages of infection foci may attain several hundred micrometers in diameter and contain thousands of pathogen cells mixed with host cell debris.

In addition to dense accumulations in foci or abscesses, the pathogen disperses throughout the connective tissue and vascular system often occluding the hemolymph sinuses. Although the epithelia are invaded and abscesses may form, epithelia are among the least damaged tissues in well-advanced infections. Adductor and smooth muscle are also invaded and extensive multiplication of the pathogen often occurs therein; however, since very heavy infections often occur, the adductor is probably not heavily damaged until late in the infection, otherwise predators would enter and kill the oyster as soon as shell closure was no longer possible.

Probably a lethal toxin is not formed by the pathogen since very large cell numbers may accumulate in all tissues before host death results. Destruction of host cells appears to be limited to those cells in the immediate vicinity of the pathogen.

ACKNOWLEDGMENTS

The author wishes to thank Deborah DeBiasi, Susan Fox, and Patsy Berry for their able technical assistance.

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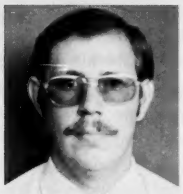
MFR PAPER 1208

Minchinia nelsoni (MSX) Disease of the American Oyster



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It has been almost 20 years since the Multinucleate Sphere X (MSX) (Fig. 1) was first recognized as the etiologic agent responsible for the sudden loss of oysters in Delaware Bay (Haskin, Canzonier, and Myhre, 1965). The first mortalities of oysters were in 1957; by the spring of 1958, massive mortalities had occurred. Losses were reported as high as 85 percent in some areas (Haskin et al., 1965). The devastation wrought by the disease was well illustrated by Sindermann and Rosenfield (1967). They indicated that oyster production in Delaware Bay prior to the epizootic was about 7.5 million pounds of shucked oyster meats. By 1960, oyster production for that area was below 100,000 pounds.

Many state, university, and Federal agencies cooperated in the search for answers to the problem. MSX was eventually identified and named *Minchinia nelsoni* (Haskin, Stauber, and Mackin, 1966). The definitive spore stage (Fig. 2) was identified and described by Couch, Farley, and Rosenfield (1966). Barrow and Taylor (1966) showed that plasmodial and spore stages shared specific antigenic properties. Ultrastructural details were described by Perkins (1968) and Rosenfield, Buchanan, and Chapman (1969). Farley (1967, 1968) proposed a life cycle for the parasite and described the disease syndrome caused by the parasite.

The disease did not remain a problem restricted to Delaware Bay. By 1959, MSX had reached the waters of Maryland and Virginia with a similar devastating impact (Andrews, 1966; Rosenfield and Sindermann, 1966; Sieling, Otto, and Rosenfield, 1969). The epizootiology of the disease in each of these areas has been thoroughly studied and reported (Haskin et al., 1965; Andrews, 1966, 1968; Rosenfield and Sindermann, 1966; Andrews and Wood, 1967; Couch and Rosenfield, 1968; Ford, 1973; Farley, 1975).

The oyster industry has made a comeback in most of the areas affected. By 1972, the disease had virtually disappeared in the Maryland portion of Chesapeake Bay, indicating a decrease in parasite pressure (Otto, Hamed, and Rosenfield, in press). In all of the areas involved, disease resistant populations have developed from the survivors of the initial epizootic (Haskin, 1974; Farley, 1967, 1975; Andrews, 1968; Haskin and Canzonier, 1969; Myhre and Haskin, 1970; Ford, 1973).

Myhre and Haskin (1970), Farley (1975), and Douglass and Haskin (In press, a) reported that the resistant oysters became infected as readily as did susceptible strains, but that resistant stocks were able to survive with the parasite and that some eventually overcome the disease. Susceptible oysters were unable to cope with the parasites and eventually died. Since Maryland oysters are no longer exposed to high parasite pressures, resistant populations could be reduced by breeding with unexposed oyster stocks, possibly rendering them more susceptible to a reoccurrence of the disease.

Farley (1968) described a hemocytic response to the parasites in susceptible oysters. There is little doubt that there is a response in susceptible oysters. The normal vesicular connective tissue is almost completely displaced by the cellular response (Fig. 3). The initial response is of the hyaline hemocyte cell type (Farley, 1967) (Fig. 4). Douglass and Haskin (In press, b) quantitatively measured the changes in the hemocyte populations due to *M. nelsoni* and found them to be from 80 to 140 percent higher than in uninfected oysters. Farley (1975) compared the hyaline hemocyte response in oysters to the T cell response in vertebrates and suggested a surveillance function for foreignness.

In resistant oysters, the infection usually involves only the epithelial cells of the gills. When the parasite does cross basement membranes and becomes established in the host, there is little host response. In later infections, the parasites appear to be moribund, some phagocytosis occurs, and the infections may eventually disappear (Farley, 1967).

The nature of the resistance mechanisms oysters have toward *M. nelsoni* is unknown. There is little doubt that resistance is involved. I have examined populations of oysters with *M. nelsoni* prevalence greater than 60 percent; however, mortalities are not comparable to those of the original epizootic. The humoral response suggested by Farley (1975) and Douglass and Haskin (In press, b) has yet to be demonstrated. Acton and Evans (1968) demonstrated enhanced nonspecific clearance of bacteriophages from the oyster. The mechanism of this enhanced memory is still in question. Tripp (1975) reviewed studies of humoral factors in molluscan immunity and indicated that there are many questions yet to be answered.

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Figure 1.—Multinucleate spherical ("X") plasmodial stage of *Minchinia nelsoni* (arrow). 1,000×.

Figure 2.—Acid-fast spores of *M. nelsoni* in tubule of digestive diverticula. Arrow indicates operculate spore (1,000×).

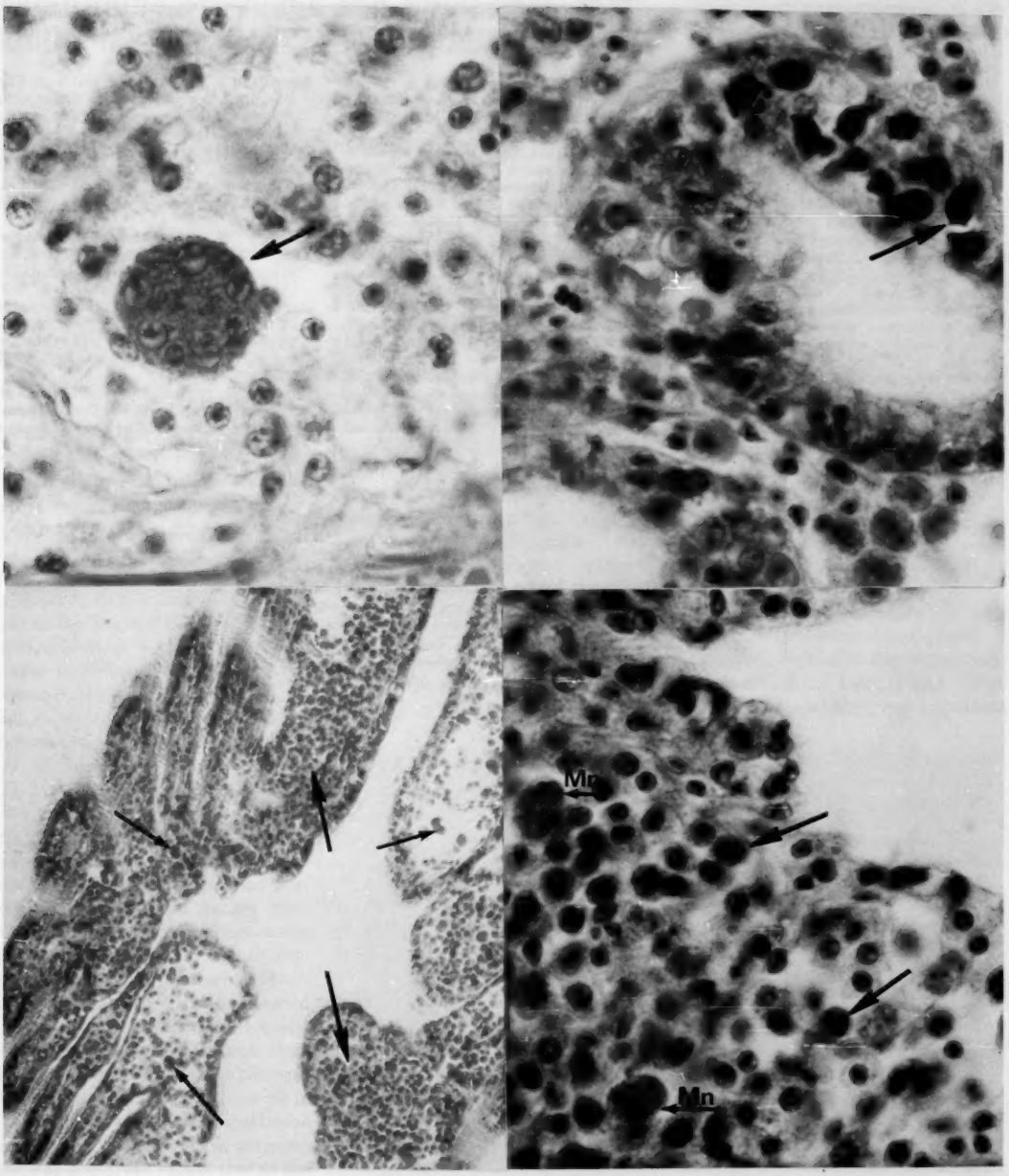

Figure 3.—Gill filament infected with *M. nelsoni* (small arrows) showing massive infiltration of hemocytes (large arrows). 200×.

Figure 4.—Higher magnification of gill lesion showing hyaline hemocyte (arrows) in response to *M. nelsoni* (Mn) infection. 1,000×.

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MFR PAPER 1209

Descriptions of Polyps and Epidermal Papillomas in Three Bivalve Mollusk Species

Harshbarger

JOHN C. HARSHBARGER

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CASE I: HYPERPLASTIC POLYP ON THE FOOT OF A FRESHWATER MUSSEL, *ANODONTA CALIFORNIENSIS*

Polypoid growths of the foot, mantle, and pericardium have been found repeatedly in bivalve molluscs. These growths (reviewed by Pauley, 1969; Sparks, 1972) generally are composed of essentially normal tissues and have been variously interpreted.

Forty-seven (15.7 percent) of 299 freshwater mussels, *Anodonta californiensis*, with foot lesions were collected by Gilbert B. Pauley from the Columbia River on the Hanford Energy Research and Development Administration Reservation in 1965 or 1966 and have been previously described (Pauley, 1967a, b, 1969). A representative specimen from this series was contributed to the Registry of Tumors in Lower Animals (RTLA 142).

The growth was 3-4 mm in diameter and extended slightly more than 2 mm beyond the surface of the foot. The gross surface, as seen with a hand lens, had a slightly sculptured texture, similar to that of the normal areas of the foot. The growth was very slightly constricted at the point of attachment.

Microscopically the growth appeared to be an extension of normal foot elements (Fig. 1). Muscle fasciculi from the normal area extended into the base of the growth and normal muscle cut in all three planes was the principal tissue throughout (Fig. 2). The basic arrangement was preserved except that as the muscle fibers fanned out from the base they were considerably further apart and the resulting space was largely occupied by fibrous connective tissue. Blood vessels were normal as regards size, frequency, and the thickness of the vessel walls. The peripheral one-fourth of the growth was heavily occupied by normal elongate glands, filled with basophilic granular product, extending up into the surface papillae (Fig. 3). The papilliferous surface was composed of a normal simple ciliated columnar epithelium (Fig. 4).

The cause of these lesions is unknown, but the basically normal arrangement of normal cell types rules out neoplasia. The two tissues that had to have proliferated to provide the extra bulk of covering were the fibrous connective tissue and the surface epithelium. The fanning out of muscle from the point of attachment suggests that muscle was pulled into the area as if an injury had caused the surface to buckle outwardly. The remaining spaces, according to this model, were then filled with scar tissue and the surface epithelium and associated gland cells proliferated normally.

In conclusion, these lesions are interpreted as traumatic polyps composed of muscle and scar tissue overlain with normal epithelium and associated gland cells.

CASE II: BENIGN MESENCHYMAL POLYP ON THE MANTLE OF A PACIFIC OYSTER, *CRASSOSTREA GIGAS*

A Pacific oyster, *Crassostrea gigas*, with a large pedunculated growth attached to the mantle was discovered in Little Skookum Inlet, Puget Sound, Wash. The specimen was contributed

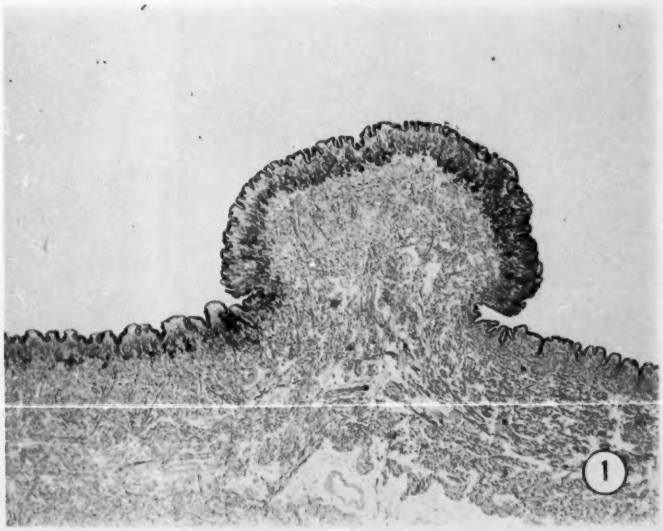

Figure 1.—Polypoid growth extending from the foot. 2.5×.

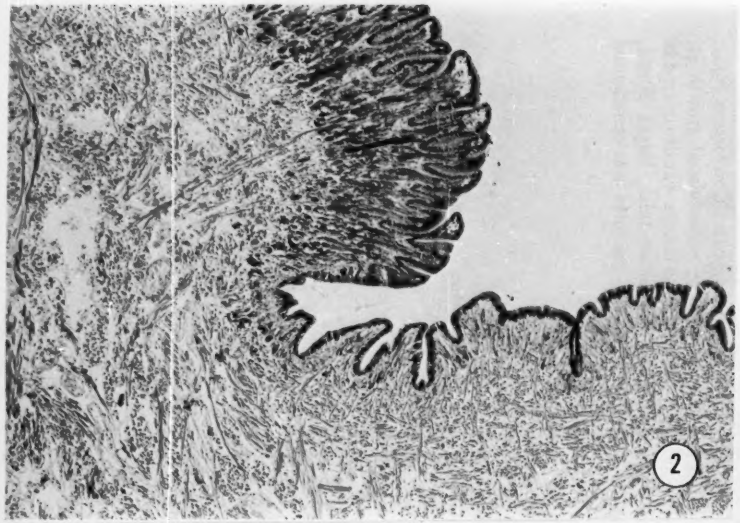

Figure 2.—Fascicul of normal appearing muscle fibers cut in all planes fan out from the base of the growth. 10 ×.

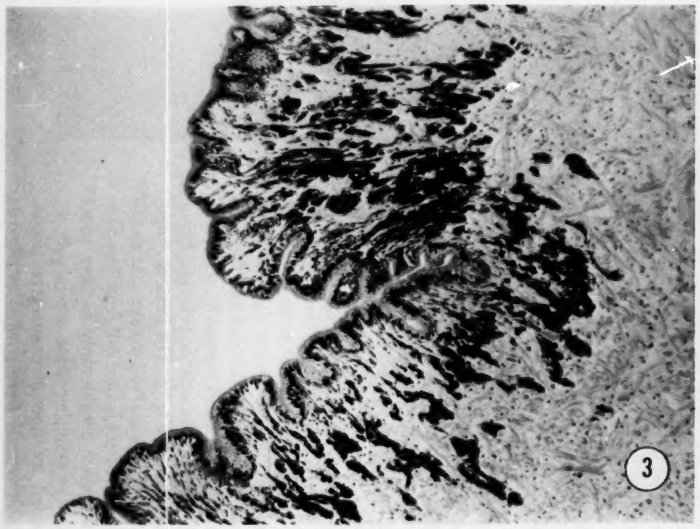

Figure 3.—Normal appearing, product filled, glands extend into surface papillae of normal and tumor epithelium. 27×.

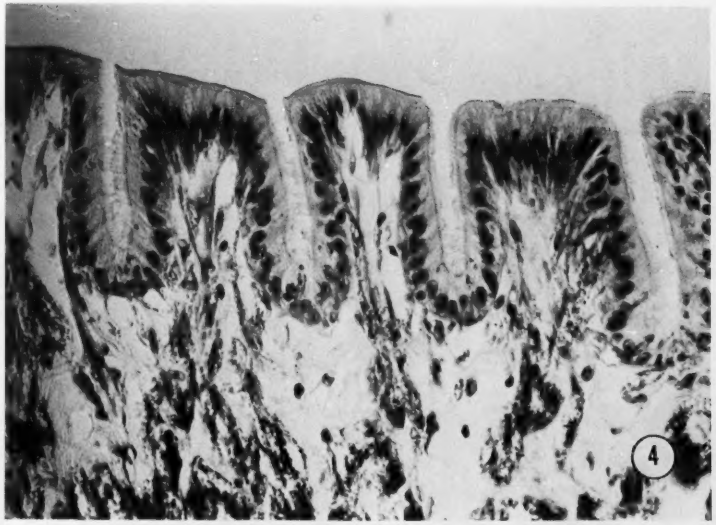

Figure 4.—Normal appearing, simple, columnar, ciliated epithelial border of tumor. 100×.

Figure 5.—Stalked polyp extending from mantle.

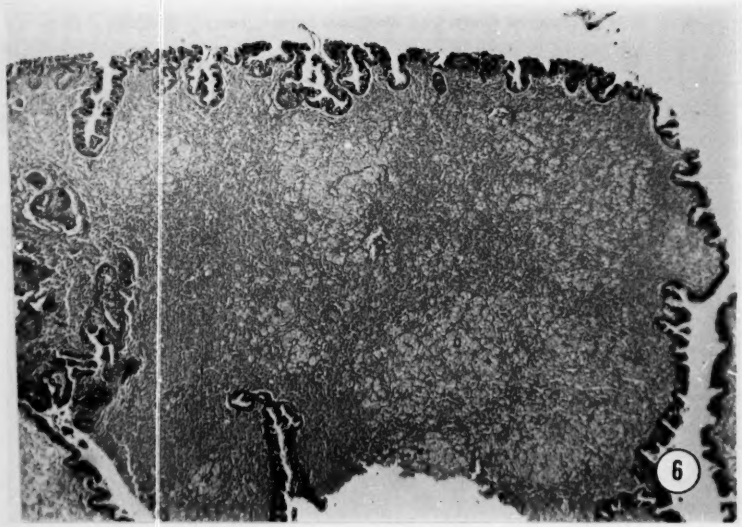

Figure 6.—Histological overview of one lobe showing Leydig component (light areas), fibrous component (darker areas), and cryptal epithelial surface. 4×.

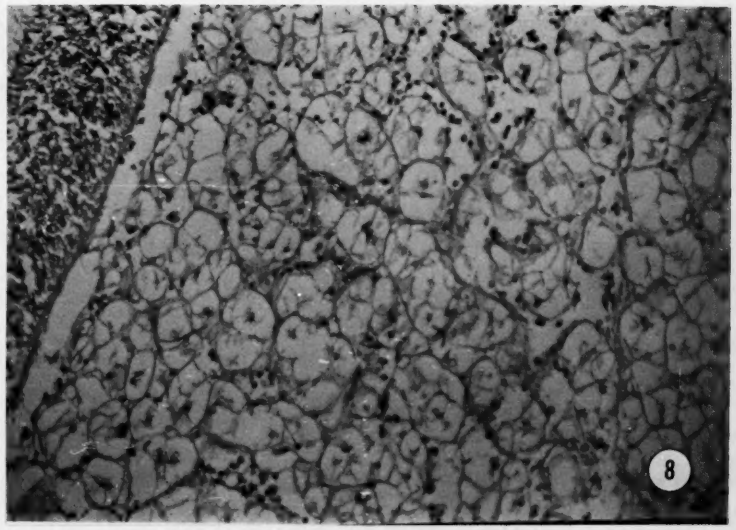

Figure 8.—Normal Leydig (vesiculated connective) tissue. 40×.

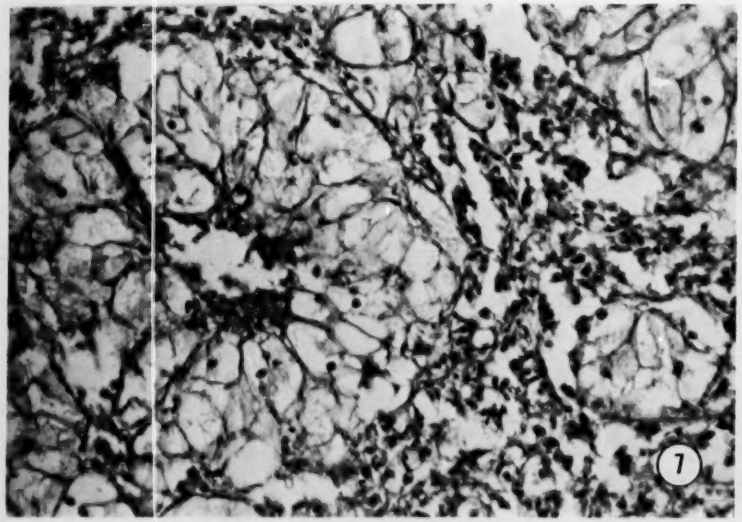

Figure 7.—Pseudorosette of Leydig tissue within fibrous stroma. 100×.

Figure 9.—Nodular growth with granular texture attached to mantle.

Figure 10.—Growth resembling clusters of grapes attached to mantle.

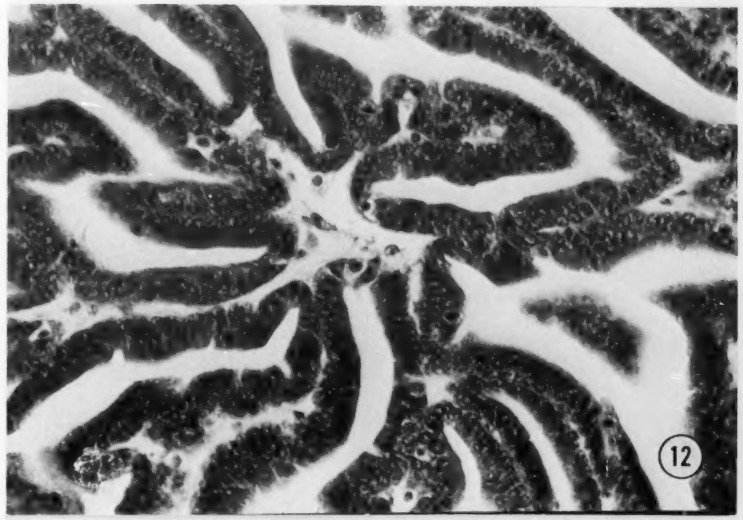

Figure 12.—Numerous mitotic figures indicate rapid growth. 160×.

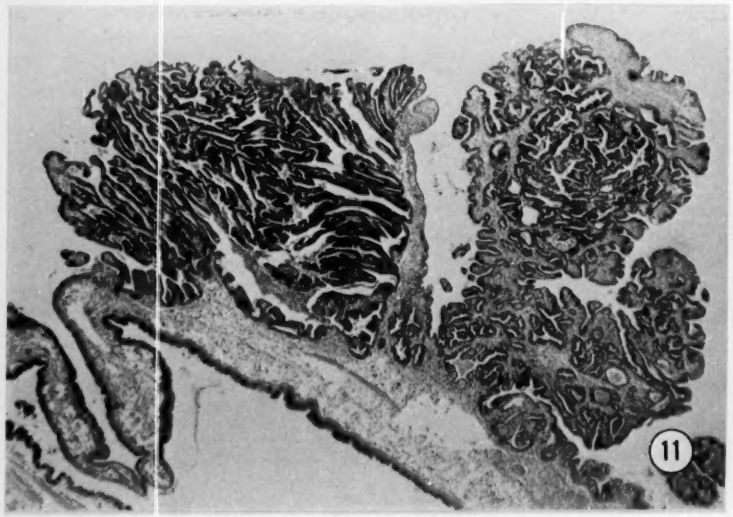

Figure 11.—Tumor nodules continuous with mantle epithelium. 13×.

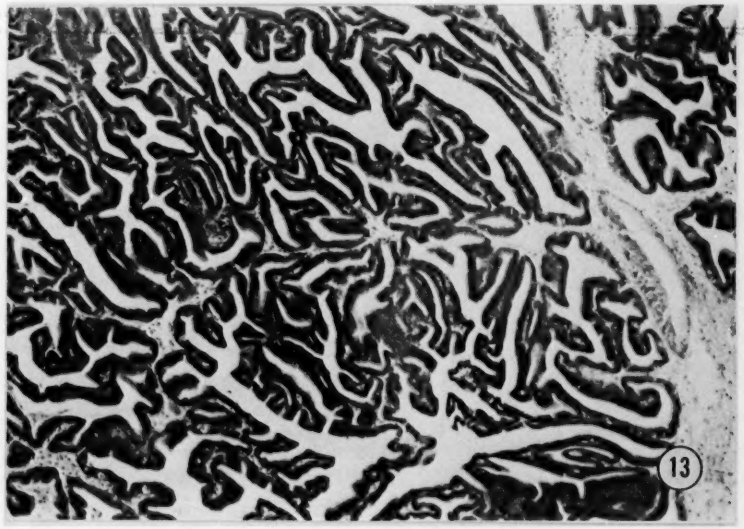

Figure 13.—Plexiform growth pattern. 25×.

to the Registry of Tumors in Lower Animals (RTLA 771) by John G. Dennison of the Olympic Research Division, ITT Rayonier, Incorporated, in March 1973. Gross and histological study showed it to be similar to lesions previously described in *C. gigas* (Sparks, et al., 1964; Sparks, Pauley, and Chew, 1969) and in *C. virginica* (Smith, 1934). The gross specimen had a firm but pliable, dull white, lobulated growth 2.5 cm in diameter attached to the mantle by a slender peduncle 1 cm in length (Figs. 5, 6). Despite the loss of some microscopic detail due to autolysis, vesicular connective tissue cells were readily discerned to comprise the major component of the growth. These cells, which were arranged singly, in bunches, and in pseudorosettes around blood vessels (Fig. 7) had tiny central nuclei surrounded by large fully expanded vesiculated cytoplasms suggesting that a large amount of glycogen had been present prior to processing. The tumor cells were indistinguishable from the normal vesicular connective tissue cells located in the usual position next to the digestive gland of the specimen (Fig. 8) although they were supported by a considerably larger fibrous stroma. The epithelial lining consisted of simple columnar cells interspersed with goblet cells similar to normal mantle except for deep crypts in some areas. Mitoses were not seen in any of the components.

In conclusion, the lesion appeared to be composed of slow-growing, noninvasive, well-differentiated vesicular connective tissue associated with abundant, possibly reactive, fibrous tissue enclosed by a simple, occasionally cryptic epithelium composed of normal appearing cells. It is interpreted as a benign vesiculo-epithelial polyp.

CASE III: EPITHELIAL PAPILLOMAS OF THE MANTLE OF ROCK OYSTERS, *CRASSOSTREA COMMERCIALIS*

During the last 11 years over 140 rock oysters, *Crassostrea commercialis*, with papillary growths arising from mantle epithelium were collected by Peter H. Wolf from several populations within the Hawkesbury and Shoalhaven River estuaries of Australia (Wolf, 1974). This is a prevalence of less than 0.001 percent which is probably low since the survey was conducted by oyster shuckers incidental to their regular duties. Both estuaries drain agricultural land, support moderate pleasure boating, and one receives effluent from a pulp mill several miles upstream but other than that, neither estuary receives industrial effluent. Limited electron microscopy has not revealed viruses. So far attempts to transplant the growths have not been successful (Wolf, 1976).

Two whole specimens contributed by Wolf to the Registry of Tumors in Lower Animals (RTLA 160 and 162) had features similar to those already described (Wolf, 1969, 1971, 1976). Grossly RTLA 162 was a nodular growth with a granular texture measuring 8 × 5 ×4 mm (Fig. 9) while RTLA 160, which was 7 × 5 mm, had the appearance of several clusters of various sized grapes (Fig. 10).

Microscopically the lesions were continuous with normal mantle epithelium (Fig. 11). However, tumor cells were more basophilic and exhibited a higher than normal nucleus to cytoplasm ratio. Mitoses were extremely numerous (Fig. 12), 28 being counted in one 400× field. This rapid growth had resulted in a random buckling which produced a plexiform pattern of paired epithelial cells separated by a thin fibrous stroma (Fig. 13). The growths were focal in nature but strands of muscle that normally underlie muscle epithelium had been drawn into the tumor confirming local invasion. Exfoliated necrotic epithelial cells associated with macrophages were abundant in some areas. These lesions were interpreted as epithelial papillomas of the mantle.

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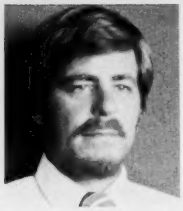
MFR PAPER 1210

Proliferative Disorders in Bivalve Mollusks

C. AUSTIN FARLEY



Farley

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INTRODUCTION

The purposes of this report are to describe briefly the types of neoplasms and related disorders seen in mollusks, to attempt characterization of their tissue origins, and to compare these diseases to vertebrate neoplasms.

METHODS

A collection of proliferative disorders in mollusks has been assembled over a period of years through pathologic studies of epizootic neoplastic disease (Christensen, Farley, and Kern, 1974; Farley, 1969b; Farley and Sparks, 1970) and routine histologic studies from many locations (Farley, 1968, 1969b; Farley and Sparks, 1970).

Diagnosis and characterization of the neoplastic diseases in mollusks discussed here were accomplished primarily by light microscopy and standard staining methods. Recently, the traditional methods have been supplemented by electron microscopy (Farley, 1976).

RESULTS

Invasive disorders have been found in 5 genera and 8 species of bivalve mollusks. Five basic types of neoplasms have been recognized, and all are thought to be invasive. Several non-neoplastic proliferative conditions have also been seen.

Non-Neoplastic Proliferative Disorders

Cellular response to the oyster pathogen *Minchinia nelsoni* is characterized by infiltration of infected tissue by hyaline hemocytes which resemble lymphocytes morphologically and produce a lesion similar to that caused by chronic inflammation in mammals (Farley, 1968, 1975). The cells involved in this parasite infection are morphologically similar to the cells involved in hematopoietic neoplasms which have been described previously (Farley, 1969a). However, in neoplasias of mollusks, evidence of parasitic involvement was lacking and anaplasia and mitotic activity was much more evident in the hemic neoplasms.

An epizootic disease was found in 6 percent of 50 clams, *Mya arenaria*, collected from Umpqua Bay, Oreg. Histologic lesions resembled hematopoietic neoplasms (Fig. 1) but appeared to be associated with the presence of small, difficult to see, protistan parasites (Fig. 2). The parasites were similar to the labyrinthuloid organisms described by Mackin and Schlicht (1976). This condition appeared to be similar to the leukemoid response seen in mammals.

Focal hyperplastic lesions of oysters, *Ostrea lurida*, were found in gill epithelia that had been introduced into Yaquina Bay, Oreg., from Washington, held in trays, and monitored for 1½ years. Lesions were seen in less than 5 percent in the base sample and in native Yaquina Bay oysters, but high prevalences (48 percent) developed in the introduced population within 3 months and continued throughout the 1½-year study. Lesions were characterized by elongation and increased cellularity of gill filaments, increased nuclear size, and increased cytoplasmic basophilia (Fig. 3). The cause of this condition was not determined.

Neoplastic Lesions

Epizootic neoplasia occurred in populations of mollusks from three areas.

Yaquina Bay Studies

The first epizootic was discovered in mussels (*Mytilus edulis*) and oysters (*O. lurida*) from Yaquina Bay, Oreg. (Farley, 1969b; Farley and Sparks, 1970). In *M. edulis*, the lesions consisted of enlarged anaplastic cells with dense lobed nuclei containing from one to five large acidophilic nucleoli. Numerous mitotic figures, many of them abnormal, were present. Lesions appeared to originate in the connective tissue spaces of the plicate organ in the mantle. Diffuse invasion of all connective tissues and blood spaces resulted in an apparently fatal outcome. The epizootic occurred locally in the lower region of Yaquina Bay with a peak prevalence of 14 percent. Lesions were seen first in August, becoming common by November, and disappearing with presumed mortality in May. A similar epizootic sarcomatoid disease was seen in *O. lurida* from Yaquina Bay (Farley and Sparks, 1970). This disease had an epizootic pattern identical seasonally and geographically to the one occurring in *Mytilus*. Lesions appeared to originate multifocally in the connective tissues of oysters. Neoplastic cells were more spherical than in *Mytilus* but had similar patterns of nuclear enlargement and density. Most contained only one nucleolus. Mitotic activity was intense only in certain phases of development. Prevalences were found to peak at 12 percent.

Other species of mollusks (*Macoma irus* and *Macoma nasuta*) from Yaquina Bay had similar anaplastic neoplastic diseases of indeterminate cytologic origin, with prevalence of 5 percent in May (Kern, personal commun.[1]). Introduced populations of mollusks (*Crassostrea gigas* and *O. lurida*) showed no evidence of neoplasia over the 1½-year study period.

Chesapeake Bay Epizootic

Epizootic gill carcinoma was found in *Macoma balthica* populations in the lower reaches of two tributaries which empty into the Choptank River (Christensen, Farley, and Kern, 1974). The disease was extremely localized geographically and had a seasonal pattern and prevalence similar to the Yaquina Bay epizootic (12 percent, October through early June). Disease was confined to *M. balthica* even though *M.*

[1]Kern, F. G. Oxford Laboratory, Middle Atlantic Coastal Fisheries Center, NMFS, NOAA, Oxford, MD 21654. Personal commun.

Figure 1.—Leukemoid response in *Mya arenaria*. HHE stain, blue stain (FPM); 312×.

Figure 2.—Protistan parasitelike cells (arrows) which stain differentially with Giemsa. 1,250×.

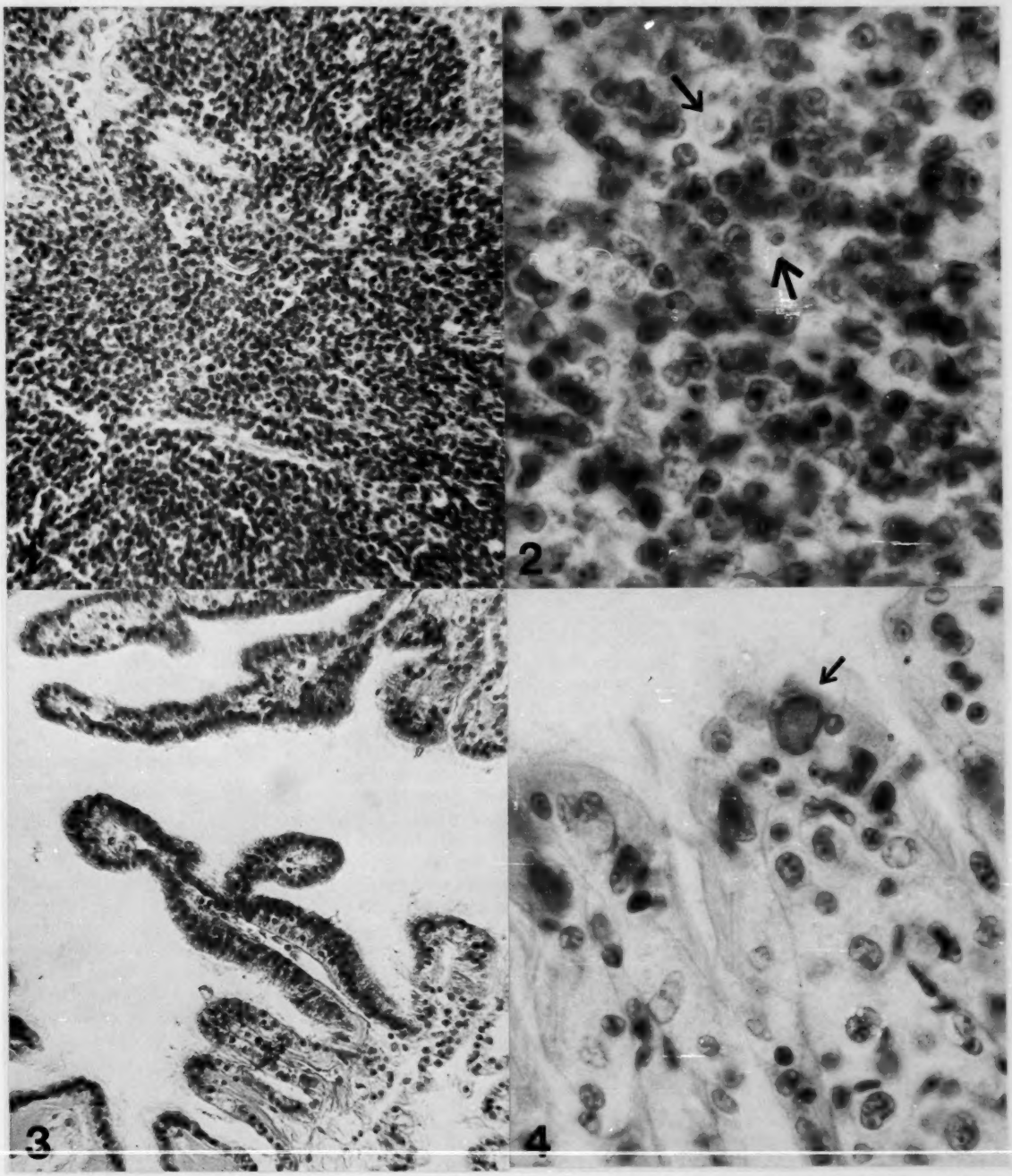

Figure 3.—Hyperplastic gill lamellae in *Ostrea lurida*; note elongation, basophilia, and increased cellularity in lesion. HHE; 312×.

Figure 4.—Feulgen positive intranuclear inclusion in gill epithelial cell from *Mya arenaria*. 1,250×.

Figure 5.—Sarcoma in *Mya arenaria* in connective tissue of gill. Feulgen picromethyl blue stain (FPM); 312×.

Figure 6.—Higher magnification of *Mya* sarcoma cells; note anaplastic appearance and mitotic figures. FPM stain; 1,250×.

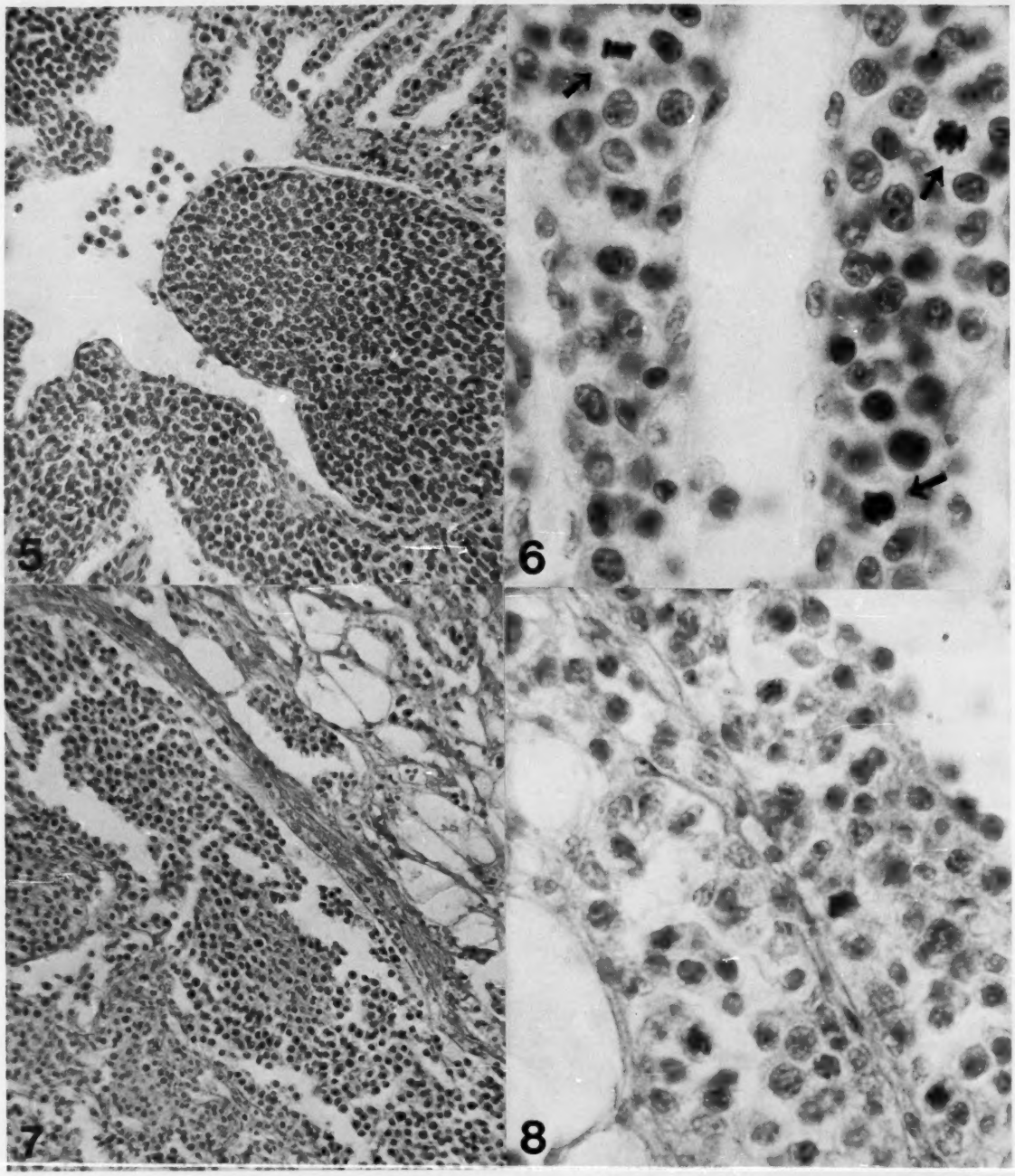

Figure 7.—Germ cell sarcoma in *Crassostrea virginica*. Neoplastic cells are in oviduct, tubules, and connective tissue spaces. FPM stain; 312×.

Figure 8.—Cytologic appearance of germinal sarcoma cells. Cells are uniform inside and anaplastic. FPM stain; 1,250×.

arenaria and *Crassostrea virginica* both occupied the same location. Histologically, the neoplastic cells were similar to those seen in *M. edulis* from Yaquina Bay. However, the primary lesions in gill epithelia suggested that epithelial cells were being transformed into anaplastic neoplastic cells. Electron microscopy confirmed that cells in the gill epithelium were transforming into frankly neoplastic cells. Fully developed neoplastic cells exhibited plical folds on the plasma membrane. The plical folding is a pathognomonic feature of these cells (Farley, 1976).

Massachusetts Epizootic

A sample of 50 clams, *M. arenaria*, was received on 19 September 1972, from Jones Creek, Annisquam River, Mass., an area where paralytic shellfish poisoning had occurred. Ten percent of the clams had gill hyperplasia; 20 percent had finely granular intranuclear Feulgen positive inclusions in gill epithelial cells (Fig. 4); and 12 percent had invasive sarcomatoid neoplasms (Kern, pers. commun., footnote one). Lesions (Fig. 5) were diffusely disseminated in connective tissue, blood spaces, and muscle. Neoplastic cells (Fig. 6) were similar to those seen in the *M. balthica* from Chesapeake Bay, with enlarged, lobed, densely granular nuclei with sparse to moderate amounts of cytoplasm. Mitotic figures were abundant.

Nonepizootic Neoplasms in Mollusks

An oyster from the Tred Avon River, Chesapeake Bay, Md., had a local lesion in the mantle consisting of anastomosing patterns of cells that were similar to cells comprising the walls of molluscan blood vessels. There were metastatic lesions in walls of blood vessels remote from the primary site. Lesions had cells with reticulin-positive external fibers surrounding them. This lesion was tentatively identified as a hemangiosarcoma by Farley and Sparks (1970).

One of 50 oysters collected on 19 September 1969, from the Mispillion River, Delaware Bay, had an advanced invasive neoplasm consisting of cells that resembled undifferentiated gametocytes and apparently were originating from the germinal epithelium (Fig. 7, 8). Diffuse invasion was seen in walls of blood vessels, connective tissue, and gastrointestinal tract epithelia. The neoplasm was diagnosed as a dysgerminoma or seminoma (Farley, unpubl. report[2]).

Newman (1972) described an undifferentiated sarcoma in an oyster (*C. virginica*) from New Haven Harbor, Conn. The sarcoma originated from a local lesion in the connective tissue of the gill. Neoplastic cells had disseminated sparsely to sites throughout the connective tissue and hemolymph spaces of the oyster. Tumor cells were enlarged and had nuclei 8-9 mμ in diamater that were characterized by dense chromatin, large multiple nucleoli, and irregular nuclear membranes. Mitotic activity was intense, with abnormal (tripolar) figures evident.

DISCUSSION

All adequately studied epizootics had several features in common. The diseases developed in the fall and continued through spring. All neoplasms were invasive, all consisted of anaplastic appearing cells, and, within a single species, the appearance of neoplastic cells was identical in each epizootic. No environmental relationships were evident nor was an etiology readily apparent. All the epizootics appeared to have a fatal outcome and prevalences within populations (roughly 12 percent) were comparable in all populations.

Comparing molluscan neoplasms, histologically, to vertebrate neoplasms demonstrates some very strong similarities as well as some distinctive differences. At this stage, it is much easier to diagnose molluscan tumors than those of vertebrates since known molluscan lesions are highly anaplastic and thus very similar to each other. Molluscan lesions closely resemble anaplastic lesions of vertebrates. It is conceivable that subtler lesions exist in mollusks but have not yet been recognized. The occurrence of lesions originating from different tissues is common to both groups, suggesting that they are biologically similar disorders. The invasive process in mollusks is different from vertebrates in that invasion is diffuse instead of metastatic. This difference is probably due to the lack of dense tissues, the semiclosed nature of the circulatory system, and the highly anaplastic nature of neoplastic cells in mollusks.

[2]Farley, C. A. Oxford Laboratory, Middle Atlantic Coastal Fisheries Center, NMFS, NOAA, Oxford, MD 21654. Unpubl. report.

ACKNOWLEDGMENT

I thank F. G. Kern for his help in diagnostic examination of many molluscan samples which in part provided cases for this study; C. Smith and D. Wright for histologic processing; and A. Rosenfield for his continuing efforts at promoting pathobiological studies at this laboratory. This work was made possible in part by FDA Interagency Agreement No. IAG-74-48(0).

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MFR PAPER 1211

Occurrence of Hematopoietic Neoplasms in Virginia Oysters (*Crassostrea virginica*)

Frierman

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E. Michael Frierman is with the Virginia Institute of Marine Science, Gloucester Point, VA 23062. This article is an extended abstract of the full paper "Occurrence of Hematopoietic Neoplasms in Virginia Oysters (Crassostrea virginica)*" by E. Michael Frierman and J. D. Andrews, published in the Journal of the National Cancer Institute, Vol. 56, No. 2, February 1976.*

The three predominant pathogens of oysters in Virginia are the haplosporidans *Minchinia costalis* and *M. nelsoni* and the protist *Dermocystidium marinum* (Wood and Andrews, 1962; Haskin, Stauber, and Mackin, 1966; Mackin, Owen, and Collier, 1950). *Minchinia costalis* is known mostly from the high-salinity seaside embayments of Virginia, Maryland, and Delaware from Cape Henry to Cape Henlopen. *Minchinia nelsoni* and *D. marinum* have wide geographic ranges and kill oysters in Chesapeake Bay and Delaware Bay where salinities exceed 15‰. These diseases have inflicted devastating mortalities upon Virginia oyster populations, and large areas of public and rented oyster grounds have been abandoned (Andrews, 1966, 1967, 1968; Andrews and Hewatt, 1957; Andrews and Wood, 1967). Extensive monitoring of oysters held in trays to study diseases (Hewatt and Andrews, 1954) and intensive sampling have provided 70 cases of a rare neoplastic disease similar to that described by Farley (1969a) and Farley and Sparks (1970).

Microscopic studies of the 70 cases found in trayed populations of oysters have not revealed a disease agent. Wide geographic occurrence with vague seasonality reduced the possibility of localized environmental causes.

The disease believed to be neoplasia of the hematopoietic system (Couch, 1969; Farley, 1969a, b; Farley and Sparks, 1970; Pauley, 1969; Wolf, 1969) had the following histologic and cytologic characteristics: 1) Atypical hyaline hemocytes characterized by enlarged nuclei, and unusual abundance of mitotic figures; 2) intensive proliferation of hemocytes; 3) connective tissues invaded and sinuses congested with hemocytes; and 4) wide range of hemocyte sizes (Figs. 1-4).

The field program at the Virginia Institute of Marine Science for studying diseases of oysters utilized "disease free" native oysters from low-salinity areas of the James River as controls. Oysters were held in iron trays lined with galvanized wire-mesh and suspended off the bottom by 12-inch legs. A zinc bar anode was attached to each tray to extend the life of the liners. Initially, 400 or more oysters were placed in each tray. Trays were distributed in major rivers to monitor monthly *M. nelsoni* activity that occurs in salinities above 15‰. Oysters were counted and cleaned of fouling every 3 or 4 weeks. Samples of 25 live oysters were prepared histologically and examined for diseases and to document their seasonality (Andrews, 1966). Dead oysters, both boxes (no meat) and gapers (meat present) were removed and gaper tissues processed. Trayed groups of known origin and history were monitored regularly from 1 to 10 years, with duration dependent on survival of oysters and the objectives of experiments.

An extensive laboratory oyster-breeding program was initiated at the Virginia Institute of Marine Science in 1964 to produce "superior genetic" strains, i.e., rapid growth, disease resistance. Many experimental lots were field monitored with intensive selection. Two inbred lots, P-58 and P-104, were found with high prevalences of hematopoietic neoplasms. Thirty-one neoplasms were found in 369 oysters from these two groups. In contrast, only 39 hematopoietic neoplasms were recorded from over 51,000 oysters examined from 1964 to 1973 in other progeny lots and native imports.

Native and laboratory-bred oysters have shown similar prevalences (0.06 percent in 36,000) from the York River. Hematopoietic neoplasms occurred in 12.5 percent of all groups of oysters from 1964 to 1973. One or more cases appeared in 11.9 percent of 134 native groups and 13.2 percent of 106 laboratory-bred groups. Only lots P-58 and P-104 exhibited relatively high prevalences.

Lot P-104 showed hematopoietic neoplasms that appeared to be associated with mortalities of oysters. A high percentage of gapers with neoplasms occurred during July 1973, and this corresponded to high mortalities and a high percentage of live-oyster infections in the absence of other diseases. Tray lot P-58 had concurrent hematopoietic neoplasms and *M. nelsoni* infections in live samples, and the presence of the two diseases precluded assignment of specific mortalities.

Neoplasms in laboratory-bred oysters have appeared as early as 217 days from spawning and as late as 880 days without clear seasonality. A case was diagnosed in a live oyster about 8 years old. In the susceptible groups, neoplasms appeared at an age of 1 year with severe mortalities. The lot P-58 cases were scattered from August 1969 through August 1970, whereas lot P-104 cases occurred mostly in July 1973 with two cases in November 1973.

A group of 100 James River native oysters was weighed weekly from 1

Figure 1.—Transverse section of *C. virginica* showing massive congestion of sinus by abnormal hyaline hemocytes. Note infiltration of the connective tissue by abnormal hemocytes. 200×.

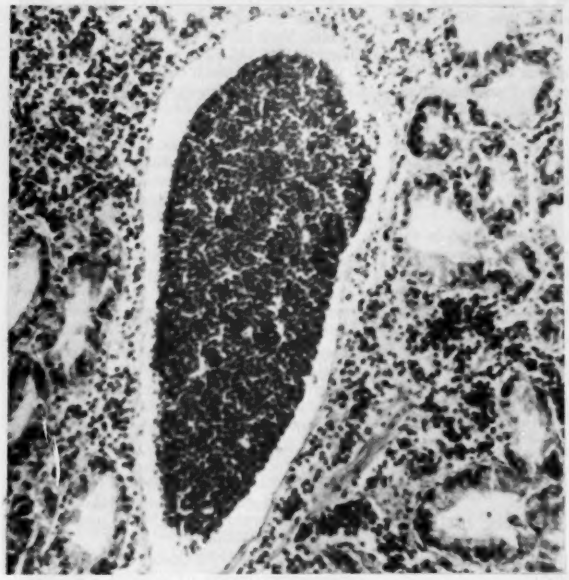
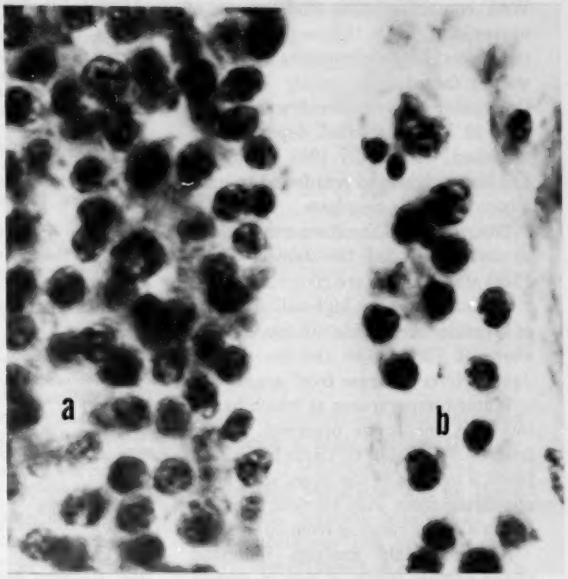
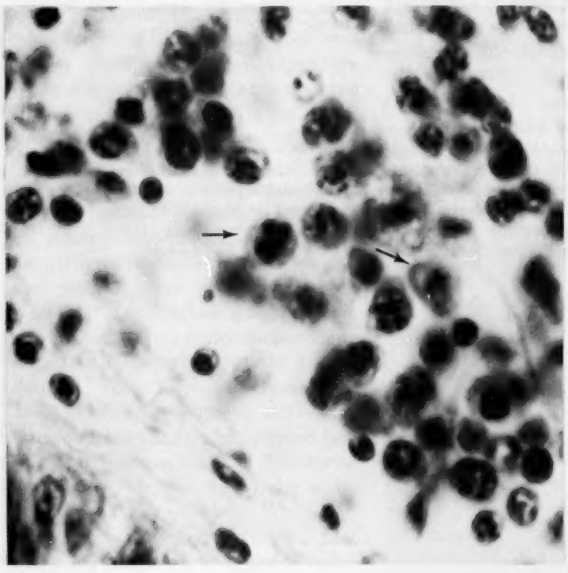
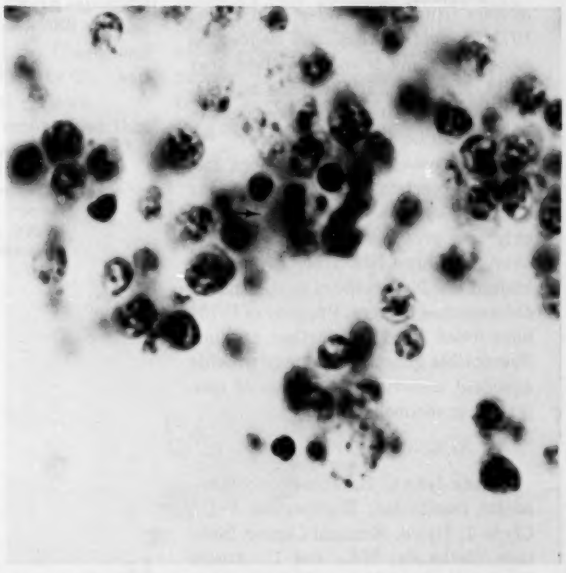


Figure 2.—Abnormal hemocytes in sinus (a) and connective tissue (b). Note enlarged, densely staining nuclei. 1,750×.







Figures 3, 4. —Abnormal hyaline hemocytes. Arrows indicate mitotic figures. 1,750×.

April 1969 by a technique of underwater weighing for growth as indicated by shell deposition (Andrews, 1961). With control of time and source of oyster importation this method permitted detection of sick oysters by lack of weight increase at weekly intervals. One oyster showed normal weight gain until 10 July when shell deposition diminished. On 17 July 1969 the oyster had lost weight and was found to have a hematopoietic neoplasm.

Distribution of the disease in Virginia estuaries included the James, York, Piankatank, and Ware rivers. No cases were found from the high-salinity areas of bayside or seaside of the eastern shore of Virginia or the low-salinity James River "disease free" area.

Water temperatures at which hematopoietic neoplasms occurred ranged from 27.9°C to 1.1°C. Cases have been found 9 months of the year, with most appearing from July through November in salinity ranging from 10 to 22‰.

Heavy metals analysis indicated above normal levels of zinc in trayed oysters (Huggett, Bender, and Slone, 1973; Huggett, Cross, and Bender, in press) resulting from galvanized tray parts. However, at this time no relationship to hematopoietic neoplasms in oysters can be ascribed to zinc.

This disease may appear more frequently in certain genetic combinations of inbred oysters. Higher incidence in only two groups suggests this, since over 100 inbred laboratory groups exhibited low incidences of neoplasms as did imported oysters. Progeny of P-104 have been bred for further studies. Susceptible genetic races may provide excellent materials for studies of neoplasms in mollusks.

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MFR PAPER 1212

A General Model for Leucocyte Cell Renewal in Bivalve Mollusks

Mix

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INTRODUCTION

It is not the intent of this paper to survey every study or discuss every hypothesis that has been formulated about blood cell renewal in bivalve mollusks; it is sufficient to state that most hypotheses have not been based on any substantial experimental evidence. Nor is it my intent to review the confusion and controversy that has circumfused molluscan blood cell terminology. I will use the term "leucocyte" when referring to molluscan blood cells except when specific cell types are being discussed (the reader is directed to Cheng and Rifkin, 1970, for an interesting discussion about the use of the term in molluscan hematology).

The purpose of this report is to describe a model of the normal leucocyte cell renewal system (CRS) that is based primarily on research conducted during the past 6 years by a number of workers. Unpublished findings from our (Mix and Tomasovic) studies using high specific activity tritiated thymidine, ^{3}H-TdR, (Mix and Tomasovic, 1973) in analyzing leucocyte renewal in *Ostrea lurida* will also be included (see Mix and Tomasovic, 1973; Tomasovic and Mix, 1974; Mix, 1975a, for a description of the methods).

It is now generally accepted that there are two common types of leucocytes found in bivalve mollusks, hyalinocytes (Foley and Cheng, 1972) and granulocytes (Galtsoff, 1964; Tripp, Bisignani, and Kenny, 1966; Farley, 1968; Cheney, 1971; Feng, et al., 1971; Ruddell, 1971a, b, c; Foley and Cheng, 1974; Cheng, 1975) (Fig. 1). Other types, fibroblasts (Pauley and Sparks, 1966, 1967; Rifkin and Cheng, 1968; Cheney, 1969; DesVoigne and Sparks, 1969; Cheng and Rifkin, 1970; Ruddell, 1971c; Sparks, 1972), fibrocytes (Foley and Cheng, 1972; Cheng, Cali, and Foley, 1974; Cheng, 1975), myoblasts (Ruddell, 1971c), and pigment cells (Stein and Mackin, 1955; Galtsoff, 1964; Haigler, 1964; Cheney, 1969; Ruddell and Wellings, 1971) have been described although there is considerable confusion and disagreement about their structure, function, and origin. For details on what is known about the structure and function of the various molluscan blood cells, see Cheng and Rifkin (1970), Sparks (1972), and Cheng (1975) for useful reviews.

Finally, it must be emphasized that the proposed leucocyte renewal model is a simple generalized model intended to describe a basic process that unquestionably occurs in all bivalves. It is obvious that variations will be (have been) found between bivalves of different subclasses and possibly even genera. Nevertheless, it is the author's judgment that sufficient studies have been conducted on enough different bivalve species to lend considerable support to the proposed model. The reader is referred to Fabrikant (1972) for a review of terminology used in this report.

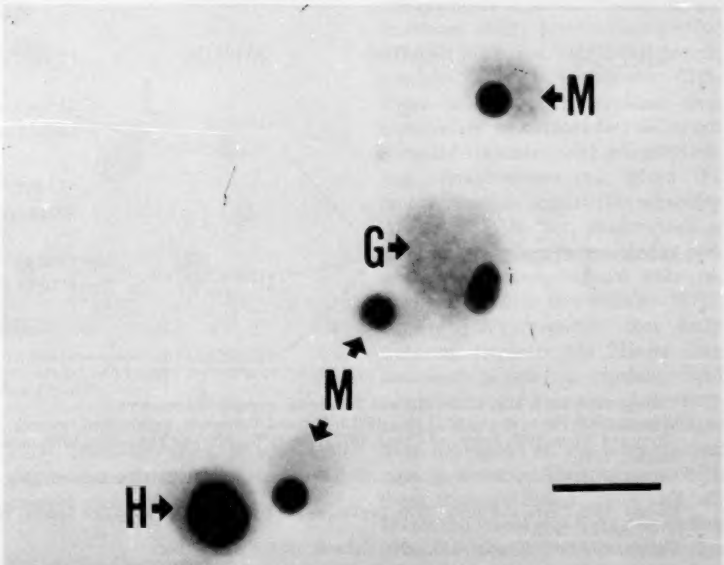


Figure 1.—Granulocytes and a young hyalinocyte from *Ostrea lurida*. G = mature granulocyte; M = young, maturing granulocyte; H = a young hyalinocyte. Bar = 5 μm.

LEUCOCYTE CELL RENEWAL

Cheney (1969), from studies utilizing H-TdR and autoradiographic analysis, proposed the following model for the development of leucocytes in the Manila clam, *Tapes semidecussata:* A hypothetical stem cell (a leucoblast or perhaps a fibroblast) divides and gives rise to a dividing population of hyalinocytes with nuclei of different diameters but usually greater than 3 μm. After one or two divisions (perhaps more) these cells enter a nondividing population and mature into phagocytes (he described only acidophilic granulocytes). He felt it was possible that the pigment cell was the final cell type and was in essence an aged acidophilic granulocyte.

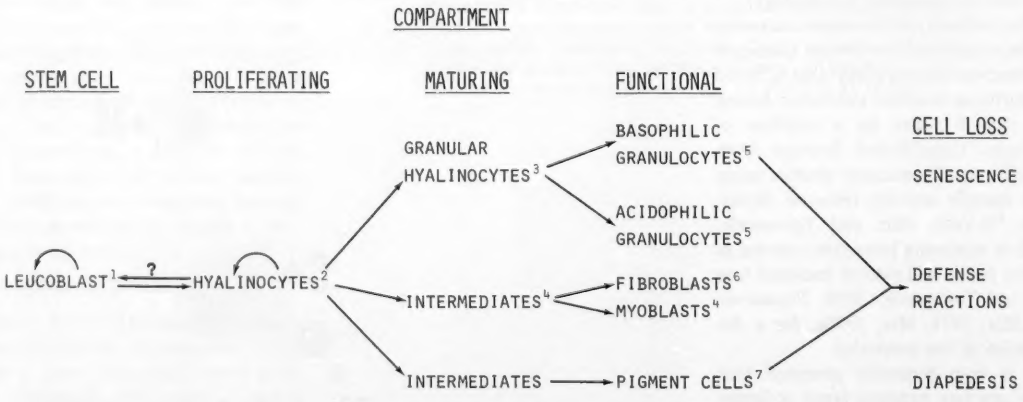
Since 1970, several studies (Cheng and Rifkin, 1970; Cheney, 1971; Feng, et al., 1971; Ruddell, 1971a, b, c; Foley and Cheng, 1972, 1974; Sparks, 1972; Mix, 1975a; Mix and Tomasovic, unpubl. report) have provided additional information about leucocyte renewal. Studies in which ^{3}H-TdR has been used (Cheney, 1969; Mix and Tomasovic, 1973; Tomasovic, 1973; Mix, 1975a; Mix and Tomasovic, unpubl. report) are, of course, particularly important. The proposed model is summarized in Figure 2. For convenience, each compartment will be considered separately.

Stem Cell Compartment

Little is known about the hypothetical stem cell. Cheney (1971) reported that the largest leucocyte in the Manila clam was similar to the vertebrate hemocytoblast and had a nuclear diameter of 6-8 μm. He felt (Cheney, 1969) that such a cell may be the stem cell, or leucoblast, of the leucocyte CRS. He also mentioned (Cheney, 1969) it was possible that a fibroblast or even a dedifferentiated gastric epithelial cell may be the stem cell but felt the latter was an unlikely source. We (Mix and Tomasovic, 1973; Tomasovic, 1973; Mix, 1975a; Mix and Tomasovic, unpubl. report) have observed large undifferentiated cells in both *O. lurida* and the freshwater mussel, *Margaritifera margaritifera,* that are consistently labeled (they are often the only cell type labeled) within hours after injection with ^{3}H-TdR (Fig. 3). This observation indicates that it probably has a relatively short cell cycle time and their frequent proximity to labeled hyalinocytes may suggest they are leucocyte stem cells. The large cells are located throughout the loose connective tissue but are most common in areas underlying the mantle. Cheney (1969) made similar observations about such cells in *T. semidecussata.* It is proposed that the large cell may be the leucocyte stem cell and suggest that "leucoblast" (after Cheney, 1969) is an appropriate term for describing it. It may be significant that there is good evidence for a single multipotential hemopoietic stem cell in vertebrates (Miale, 1972).

Proliferation Compartment

There is little doubt that hyalinocytes are members of the P compartment (Fig. 4) and mitotic division of these cells serves to amplify the number of cells which enter the M compartment (Cheney, 1969; Feng, et al., 1971; Ruddell, 1971b; Mix, 1975a; Mix and Tomasovic, unpubl. report). Interestingly, this possibility was described at the beginning of this century (Kollman, 1908). It is not known how many divisions occur before the hyalinocyte enters the M compartment. Two factors inhibit the resolution of this problem: The ^{3}H-TdR label may



[1] Cheney, 1969, 1971; Mix, 1975b; Mix and Tomasovic, unpublished research.
[2] Cheney, 1969; Feng, et al., 1971; Mix, 1975b; Mix and Tomasovic, unpublished research; Tomasovic, 1973.
[3] Cheng and Rifkin, 1970; Foley and Cheng, 1972; Mix, 1975b; Mix and Tomasovic, 1973, unpublished research; Tomasovic and Mix, 1974.
[4] Ruddell, 1971c.
[5] Cheney, 1971; Farley, 1968; Feng, et al., 1971; Foley and Cheng, 1972, 1974; Galtsoff, 1964; Mix, 1975b; Ruddell, 1971a, b, c; Tripp, et al., 1966.
[6] Cheney, 1969; Cheng and Rifkin, 1970; DesVoigne and Sparks, 1969; Pauley and Sparks, 1966, 1967; Rifkin and Cheng, 1968; Ruddell, 1971c; Sparks, 1972.
[7] Cheney, 1969, 1971; Cheng, et al., 1974; Galtsoff, 1964; Haigler, 1964.

Figure 2.—A simple, generalized model of bivalve molluscan leucocyte renewal. Arrows indicate the flow of developing cells from one compartment to the other. Footnotes refer to appropriate references.

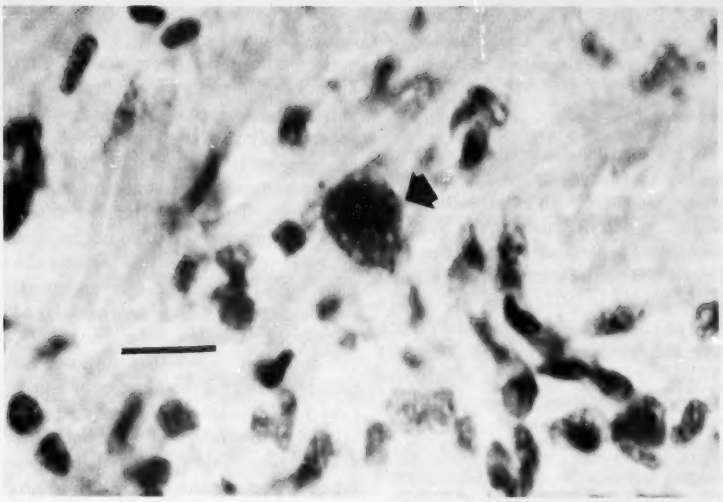


Figure 3.—A large leucocyte, thought to be a leucoblast labeled with tritiated thymidine, in the connective tissue underlying the mantle in *O. lurida*. The developed grains have obscured the nuclear details. Bar = 10 μm.

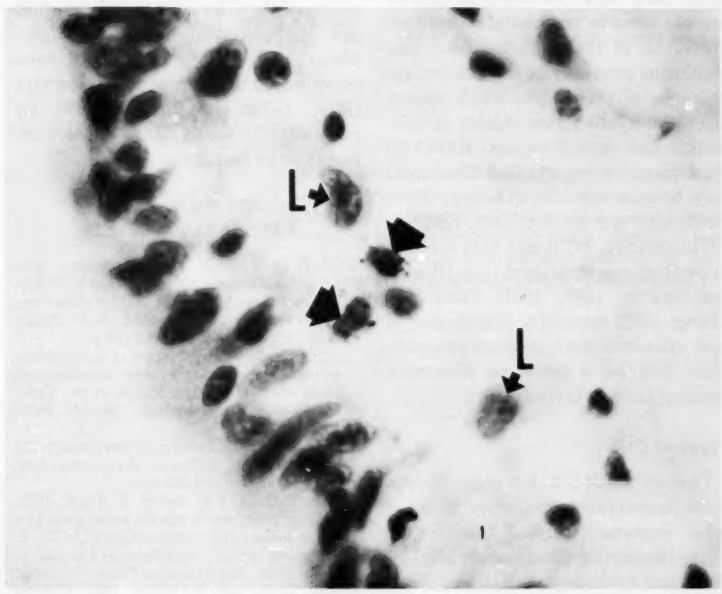


Figure 4.—Normal *O. lurida* hyalinocytes (arrows) labeled with tritiated thymidine 24 h after injection. L = leucoblasts (see Fig. 1).

become too diluted after two or three divisions, and if the bivalve has been stressed, hyalinocytes may enter the M compartment without dividing; such a mechanism is well known in vertebrate leucopoiesis. The only other candidate for inclusion in the P compartment may be the fibroblast (Cheney, 1969). However, there is very little evidence available at this time to support such a view.

Maturation Compartment

In any cell series, an almost infinite gradation of cells exists between the most immature blast cell and the mature definitive forms. These intermediate types have undoubtedly been responsible for a considerable amount of disconcertion in evaluating molluscan leucocyte structure. Thus, slightly granular hyalinocytes (Cheng and Rifkin, 1970; Foley and Cheng, 1972), cells with morphological gradations between hyalinocytes, fibroblasts, and myoblasts (Ruddell, 1971c), immature basophils (Ruddell, 1971b), basophilic granular hyalinocytes (Cheng and Rifkin, 1970) and pigment cells "forming in leucocytes" (Stein and Mackin, 1955) logically seem to constitute cells in the M compartment. An example of the confusion caused by intermediate cells is illustrated by a report (Ruddell, 1971c) that *Crassostrea gigas* hyalinocytes were observed phagocytosing various materials during wound repair. This statement has not gone unchallenged (Foley and Cheng, 1972) since results from other earlier studies had led to the conclusion that only granulocytes possess the ability to function as phagocytes (e.g., Galtsoff, 1964; Cheng and Rifkin, 1970; Sparks, 1972). A possible explanation is that since the "phagocytosing hyalinocytes" were responding to trauma, they were able to phagocytose materials prior to completing differentiation to a granulocyte. Reports of immature cells having functional ability prior to completing differentiation are not uncommon in carefully studied vertebrate CRS. There is also ample evidence that mammalian hematopoietic cells not normally associated with phagocytosis (e.g., lymphocytes) can, given the proper stimulus, acquire this capability (Miale, 1972). In fact, phagocytosis is said by some to be a potential of all vertebrate reticulo-endothelial cells regardless of their fate (Miale, 1972). Cheney (1969) reported that early maturing forms in the Manila clam possessed phagocytic capability and concluded that the rates of development in maturing and functional phases were dependent on the intensity and type of stimulus. Thus, since in both these studies (Cheney, 1969; Ruddell, 1971c) the leucocytes were responding to insults (wounds and burns), it seems likely that, given a sufficient stimulus, differentiating leucocytes may become functional before they are fully mature.

Functional Compartment

Granulocytes

Virtually all studies on bivalve leucocytes indicate that granulocytes are completely differentiated, functional cells with a finite life span; such cells in carefully studied CRS are not capable of dividing. However, Cheng and Rifkin (1970) concluded, after surveying the older literature, that mature molluscan leucocytes are capable of dividing. Not only is this inconsistent with what is known about CRS, but studies in which ^{3}H-TdR has been employed (Cheney, 1969; Tomasovic, 1973; Mix, 1975a; Mix and Tomasovic, unpubl. report) show rather conclusively that division of mature granulocytes does not occur. Labeled granulocytes are not found until several days or weeks after ^{3}H-TdR injection which strongly suggests that they are formed via differentiation pathways of other cell types and not by mitosis of preexisting granulocytes.

Foley and Cheng (1972) described *C. virginica* granulocytes that contained basophilic, acidophilic and/or refractile granules. From this observation, they concluded that the three types of granulocytes represented different stages in the life span of one cell type. There seems to be little evidence to support this hypothesis. It seems more likely that there is more than one terminal type of granulocyte for the reasons outlined below. Ruddell (1971a) had ascribed rather precise functions to basophilic and acidophilic granulocytes in *C. gigas* which suggests that these are fully differentiated cells. Both immature acidophils (Cheney, 1969) and basophils (Ruddell, 1971a, b, c) have been described in bivalves. Finally, when one considers the maturation sequence of vertebrate granulocytes, there is no a priori reason to believe that mixtures of granules are indicative of different developmental stages of a single cell type. When cytoplasmic granules first appear in vertebrate granulocytes, they are few, coarse, and wine red. The number gradually increases and the granules differentiate into three types and in the mature cells, one of these types will predominate: Acidophilic (eosinophils), basophilic (basophils), or in neutrophils, both granules are present (Miale, 1972). Clearly, additional studies are required to resolve the question of granulocyte development.

Fibroblasts

Much remains to be learned about molluscan fibroblasts which are normally associated with inflammation and wound repair (Pauley and Sparks, 1966, 1967; DesVoigne and Sparks, 1969; Cheng and Rifkin, 1970; Cheney, 1971; Ruddell, 1971c; Sparks, 1972). Sparks (1972) has reviewed the structure and function of this cell.

There are several reports of hyalinocytes differentiating or maturing into fibroblasts (Pauley and Sparks, 1966, 1967; Rifkin and Cheng, 1968; Des Voigne and Sparks, 1969; Cheng and Rifkin, 1970; Ruddell, 1971a, c; Sparks, 1972) at various times after inflammatory responses are initiated and the autoradiographic studies (Cheney, 1969; Mix and Tomasovic, unpubl. report) seem to corroborate these reports. All of these studies base that conclusion primarily on the delayed appearance of these cells which appear after the hyalinocytes during inflammation and wound repair. However, since there are reports that fibroblasts may be pluripotential (Cheney, 1969; DesVoigne and Sparks, 1969; Ruddell, 1971c; Sparks, 1972) and that there is more than one structural type (Pauley and Sparks, 1966, 1967; Foley and Cheng, 1972) means the installation of such cells into the functional compartment may be a tentative placement pending further investigation.

Pigment Cells

Cheney (1969) felt that pigment cells were degenerative granulocytes while other reports described their origin from leucocytes (Stein and Mackin, 1955) and hyalinocytes (Haigler, 1964). Ruddell and Wellings (1971) felt that pigment cells of *C. gigas* were possibly involved in the transport and processing of biological fluids which would indicate that they belong in the F compartment.

Finally, as in all CRS, leucocytes are lost from the F compartment by various routes including senesence, diapedesis, or defense reactions ("death in the line of duty" [Cronkite et al., 1959]).

The proposed leucocyte CRS model is the result of attempts to draw together information from studies that have been directly or indirectly involved with leucocyte renewal. It seems likely that the above model will be completely accurate in some respects and perhaps totally inaccurate in others. There are myriad unanswered questions which do not require listing here since most will occur to the knowledgeable reader. Nevertheless, the ultimate function of any model is to stimulate further inquiry and hopefully, this model will serve that purpose. An understanding of the kinetics of leucocyte proliferation is obviously a prerequisite to discerning not only the normal steady state renewal but also for interpreting the modalities of abnormal proliferation associated with diseases and responses to different environmental insults. Our nescience on this subject precludes understanding molluscan proliferative disorders which are suggestive of sarcomatoid neoplasia; such abnormalities have been described in this volume and elsewhere (Mix, 1975a, b; Pauley, 1969 for reviews). Obviously, a great deal remains to be learned.

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MFR PAPER 1213

Gonadal and Hematopoietic Neoplasms in *Mya arenaria*

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The reproductive tract of the soft shell clam, *Mya arenaria*, goes through a number of morphologic changes during its reproductive cycle (Coe and Turner, 1938). While studying several thousand animals from all coastal areas of the United States, many variations were encountered in the "normal" pattern of gonadal development. Animals, collected from two areas in the State of Maine which are known to have been contaminated by oil, exhibited abnormal cell development.

At Long Cove, Searsport, Maine, there was an oil spill in 1971 of #2 fuel oil and the jet fuel, JP-5 (Mayo, et al.[1], 1972). There has been a chronic seepage of oil since then. Microscopic examination of *Mya arenaria* collected from this site during the last 4 years revealed neoplastic growth possibly originating from germ cells in the gonadal tissues of both male and female clams. Neoplastic lesions in the gonadal tissue of the soft shell clams contained cells with mitotic figures, pleomorphic mononucleated cells, and multinucleated giant cells. Many nuclei were lobed and irregular containing two or more prominent red-staining nucleoli and were hyperchromatic with clumping of nuclear chromatin.

In animals in which normal tissue

[1]Mayo, D. W., C. G. Cogger, D. J. Donovan, R. A. Gambardella, L. C. Jiang, and J. Quan. 1972. Correlation of spilled oil in the Searsport, Maine, region by gas chromatography. Bowdoin College, Department of Chemistry, Brunswick, Maine. November 1972. Tech. report (unpublished ms.), p. 3.

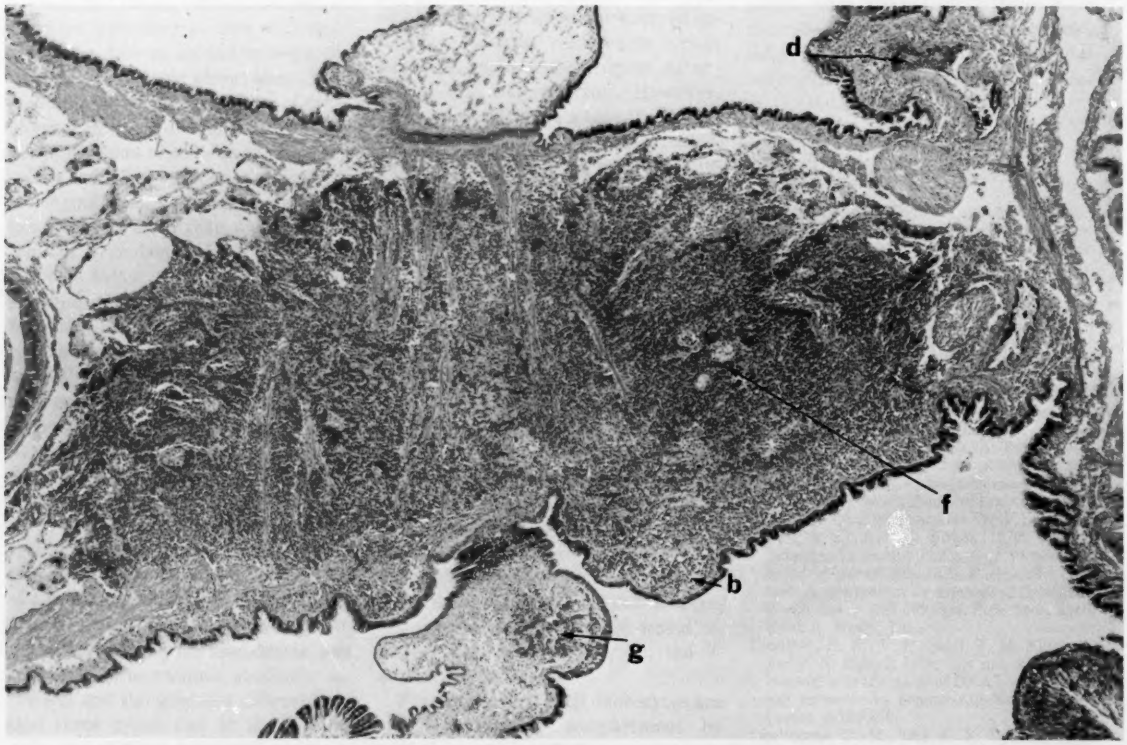

Figure 1.—Soft shell clam, *Mya arenaria*, collected from the oil spill site, Searsport, Maine. Gonadal tumor. Note extensive invasion by neoplastic cells throughout the body. Arrows point to specific areas of invasion. Key: f = follicles and connective tissue of the gonadal area; b = body wall; d = genital duct; and g = gill.

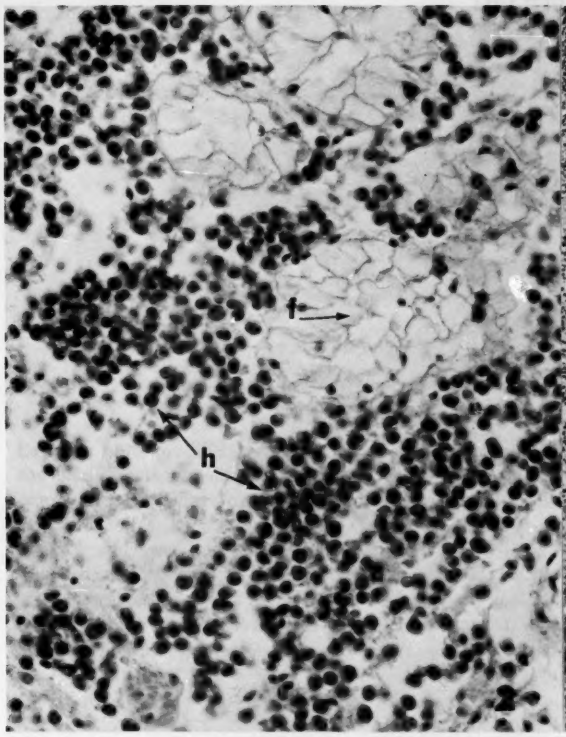

Figure 2.—Soft shell clam, *Mya arenaria*, collected from oil spill site, Harpswell, Maine. Hematopoietic tumor. Neoplastic cells occupy connective tissue areas of the reproductive tract. Arrow h indicates hematopoietic tumor cells. Arrow f indicates gonadal follicles.

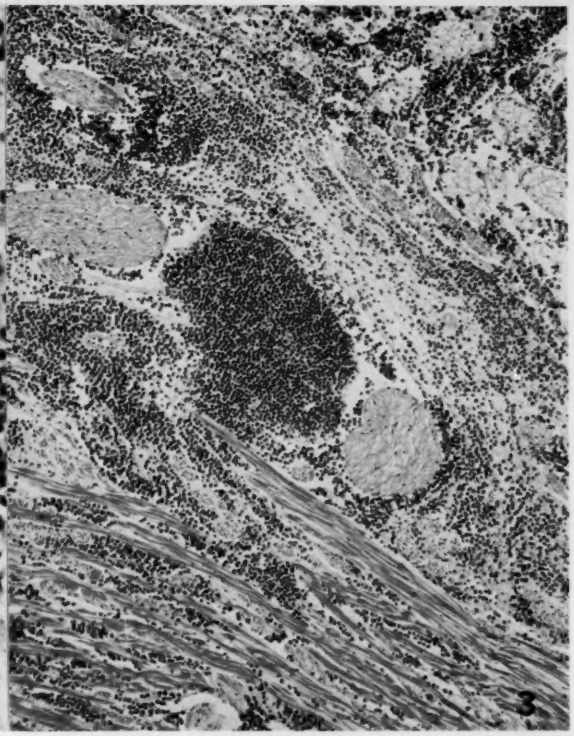

Figure 3.—Soft shell clam, *Mya arenaria*, collected from an oil spill site, Harpswell, Maine. Hematopoietic tumor. Note invasion of neoplastic cells between the muscle bundles of the foot.

structures were still evident, the gonadal characteristics appropriate to the sex of the clam were seen. However, in some instances, the neoplastic development was so extensive that it replaced the normal gonadal tissue, making sexual differentiation by histologic examination difficult or impossible. Some of the soft shells showed invasion of the interfollicular connective tissue of the gonadal area, body wall, efferent blood vessel of the gills, epibranchial chamber, genital and urinary pores, kidney, pericardial wall, heart, and red gland by the neoplastic cells. Eventually the normal architecture of these areas was destroyed (Fig. 1).

At the site of the Naval Reservation tank farm, Freeport, Maine, known as the Brunswick or Harpswell oil spill site, a seepage of jet fuel, JP-4, from the tank area has contaminated adjacent clam flats for several years. Neoplastic growth was observed in soft shell clams collected from this site, but the site of origin and type of tumor was different than that in animals from Long Cove.

These cells probably originated in the mesenchymal cells of the body connective tissues and appear to be of hematopoietic lineage. The cells possess large nuclei which may be pleomorphic or lobed (Fig. 2). Some show numerous and sometimes bizarre mitotic figures. The cytoplasm of these cells may contain fine red granules. There are extensive accumulations of neoplastic cells in the gills, siphon, foot, and in connective tissues throughout the body (Fig. 3).

No tumors similar to those just described have been encountered in animals collected from any other area. At present it is not understood why tumors consisting of morphologically different cell types are encountered in the two areas. The development of tumors does not appear to be associated with any seasonal or cyclic changes. It is more probable that they are the result of a factor or factors in their environment. Very little is known about the hydrocarbon concentration, water, sediment, and water column at these sites, and more importantly, of the reaction of the soft shell clam to the wide variety of hydrocarbons that constitute fuel oils.

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Dermal Lesions and Amebocytic Accumulations in Snails

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Pathology in mollusks such as *Biomphalaria glabrata* is sometimes accompanied by activation of the hemopoietic organ in the pericardial wall (Lie, Heyneman, and Yau, 1975; Richards, 1975). Hemopoietic activity has been observed in *B. glabrata* with dermal lesions and with amebocytic accumulations.

During studies on the genetic variation in susceptibility of *B. glabrata* for infection with *Schistosoma mansoni* much had been learned about the role of genetics in other infections and pathologic conditions. Two types of dermal lesions have been observed in different stocks of *B. glabrata*. Experimental studies suggest that each of these pathologic conditions involves an infectious agent and genetic susceptibility. Although lesions are remote from the heart, the hemopoietic organ in the pericardial wall is activated.

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The occurrence of proliferative amebocytic accumulations in four different clonal stocks of *B. glabrata* was reported by Richards (1975). The conditions in the four snail stocks have common features: occurrence of the amebocytic accumulations is genetically regulated; the accumulations originate as aggregations of amebocytes; amebocytic infiltration of hemolymph sinuses around stomach, intestine, albumen gland, digestive gland, and in the kidney may occur persistently; and the hemopoietic organ in the pericardial wall becomes enlarged in production of amebocytes. In vivo manifestation, supported by genetic experiments, demonstrated consistently different patterns of amebocytic accumulations in the four snail stocks. One type involves atrial nodules (Fig. 1) which first appear shortly after onset of egg laying, increase for a few weeks, then usually regress and disappear. In a second type, after maturity, accumulations appear in the pericardial cavity and develop progressively thereafter (Fig. 2). In a third type nodules appear around the aorta after maturity. In the fourth type nodules appear in juvenile snails on and between the loops of the intestine (Figs. 3, 4).

Selection and selfing through several generations in all four clonal snail stocks has resulted in amebocytic accumulations in 50-100 percent of the snails. Crossing experiments suggest that each of the four types of amebocytic accumulations is determined by a complex of genetic factors, and that each type is genetically different

Lie, et al. (1975) reported acquired resistance to infection in *B. glabrata* exposed first to irradiated echinostome miracidia and then challenged with normal echinostome miracidia. Following the challenge exposure the hemopoietic organ rapidly became much enlarged but returned to normal a few days after destruction of the parasites. In the case of the dermal lesions and amebocytic accumulations, which developed slowly but persistently, enlargement of the hemopoietic organ (Fig. 2) occurred more slowly and to a lesser extent but persisted longer. When the amebocytic accumulations are transferred to tissue culture media, the cells separate and form a monolayer of motile amebocytes. Although cell division has not been observed, some of the amebocytes survived and remained motile for several months in vitro. Since the effective life span of *B. glabrata* is about 1 year, it would appear possible that some amebocytes may survive for the life of the snail. If the primary function of these amebocytes is defense against infection or injury, then in the absence of such stress there may be little need for hemopoietic activity. When the hemopoietic organ is activated, the abruptness, magnitude, and duration of its activity probably depends on the nature of the stress to the snail.

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Figure 1.—Amebocytic accumulation (arrow) in atrium (A) of *B. glabrata*; kidney (K), pericardial cavity (C); 100×, Gomori's trichrome.

Figure 2.—Amebocytic accumulations (arrow) in pericardial cavity (C) of *B. glabrata*; also showing hemopoietic organ (H) in pericardial wall (P), atrium (A), pulmonary cavity (L); 100×, hematoxylin and azure-eosin.

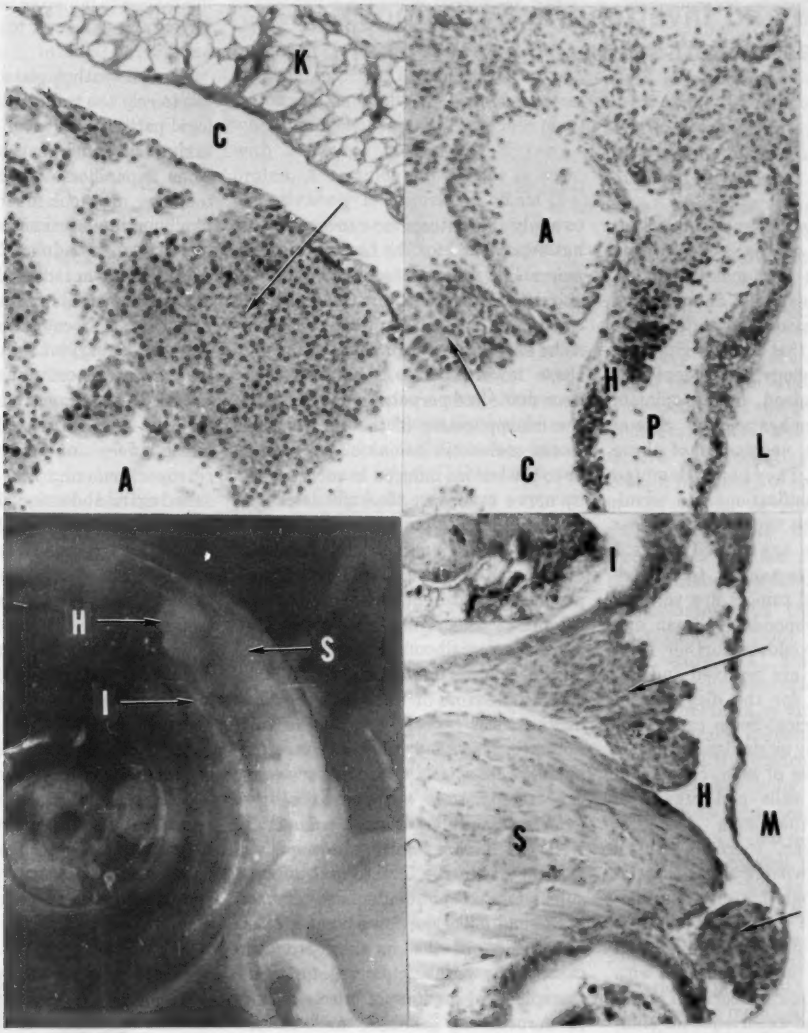

Figure 3.—Left side of live *B. glabrata* showing amebocytic accumulation in hemocele (H), stomach (S), intestine (I); 12×.

Figure 4.—Amebocytic accumulations (arrows) in hemocele (H) of *B. glabrata*; stomach muscle (S), intestine (I), mantle cavity (M); 100×, hematoxylin and azure-eosin.

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MFR PAPER 1215

Some Observations on Comparative Vertebrate and Invertebrate Pathology: A Summary Discussion of the Workshop

HAROLD L. STEWART

While there are some similarities among cancers of the same site in different species of domestic, laboratory, and captive wild animals and birds, there are also many differences. A point of view that many writers on comparative oncology have adopted is to emphasize, indeed, in my opinion, often to overemphasize, the resemblances between neoplasms of these animals and man. They have attempted to adapt the classifications and terminologies of human neoplasms to those of animals. This has misled investigators, in some instances, to believe that many animal tumors are suitable models for corresponding human diseases. Nothing could be further from the truth. There are no fixed and immutable criteria for the diagnosis of cancer in all species. Some cancers of lower animals may be similar and others dissimilar to those of man. Some cancers arise from cells and organs of lower animals that man does not possess. Moreover, there is still a hiatus in our knowledge about correlations of morphologic patterns and biologic behavior of certain neoplasms, some neoplastic-like conditions, and some lesions that may be confused with neoplasms. It required scores of years of observation and experience for clinical, veterinary, and experimental pathologists to develop the sophisticated knowledge about these subjects that now exists. But the troubles that beset the pathologist in his studies of cancerous and allied diseases in the vertebrates pale, by comparison, when investigators embark on studies of pathologic material of invertebrate species.

The anatomy, histology, and physiologic functions of these lower species confuse the oncologist as he attempts to apply the criteria for cancer that he has established for the higher forms of animal life. As Dawe[1] said at this workshop, the question "What is cancer?" constantly confronts the investigator as he examines abnormalities of form in these lower species. Mistakes have been made and perpetuated, as witness the misapplication of the term cancer to the melanotic lesions of *Drosophila* or to the lesions induced in cockroaches by nerve severance. In human beings, too, comparable mistakes have been made as, for example, the misdiagnosis of the non-neoplastic epidermoid hyperplasia surrounding insect bites of the skin or the dermal lesions of blastomycosis as carcinoma. There is a whole host of lesions of laboratory animals that at one time or another were misdiagnosed as cancer, lesions which do not progress beyond the stage of simple hyperplasia. Among these are the adenomatous hyperplasia of the gastric glandular mucosa of strain NHO and strain I mice, the papillary acanthomas of the forestomach of rats subsisting on an inadequate diet of bread and milk, the polypoid adenomatous lesion of the monkey stomach caused by a parasitic worm, squamous metaplasia of the lung of mice exposed to aerosols of gasoline, and adenofibrosis of the liver of rats, a self-limiting lesion that heals by scarring.

Despite the histologic resemblance of these lesions to cancer at some stage in their evolution, they do not infiltrate the surrounding tissues, and do not metastasize, and where feasible to test, do not transplant to syngeneic animals. The lesson that these experiences teach us is that appearances may be deceptive and mislead the observer. I would predict that with a newly emerging science like the pathologic study of cancer in mollusks, similar mistakes are bound to happen but to be forwarned will tend to minimize their number.

Clinical pathologists have learned not to rely too confidently on the histologic pattern of a given lesion without taking into consideration other factors. Thus a papilloma of the larynx in a teenager may look much the same as a papillomatous lesion of the urinary bladder of an adult. Yet the former disappears spontaneously, whereas the latter, if untreated, pursues a malignant course. Local excision will cure a benign looking, smooth muscle tumor lying in the subcutis of a patient, but a tumor looking exactly the same histologically may recur and metastisize if located deep in the thigh or in the retroperitoneum. Likewise the so-called extra-abdominal desmoid tumors of women are considerably more likely to infiltrate locally and to recur following resection, than are the histologically similar tumors of the abdominal wall.

These examples demonstrate the biological influence of location on lesions having a similar histologic appearance. Although most neoplasms exhibit pronounced departures from the histologic pattern of the normal organ, thyroid gland carcinoma may prove to be an exception: a metastatic deposit of thyroid gland carcinoma in bone may be indistinguishable histologically from a section of the normal gland in the neck.

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Some cancers emerge from a milieu of inflammatory processes, as for example Kaposi's angiosarcoma; and other cancers from non-neoplastic cellular proliferations that persist for long periods of time, such as leukemia in patients suffering from Sjögren's syndrome. There are, in addition, debatable lesions that occupy a never-never land or have a shaky foot in two camps as exemplified by reticulosis, or Letterer-Siwe disease, which some regard as a metabolic process, others as a neoplasm.

The appearances of the early developing stages of many cancers are so innocent as to be disarming. This is true of alveologenic carcinoma of the lung of mice and rats, trabecular carcinoma of the liver of the same species, adenocarcinoma of the uterine corpus of the rabbit, and papillary carcinoma of the urinary bladder of man.

Using laboratory animals it has been possible to design carcinogenic experiments involving serial killings and serial sectioning of the affected site, to trace the origins of cancer to a single cell type, as for example the type II alveolar cell that comprises the alveologenic carcinoma of the mouse. One can, as Farley[2] told this workshop, take advantage of nature's experiment, where epizootics of cancer are arising in mollusks in a confined area, to trace the cancer back to its cell or cells of origin. As shown at the workshop, electron microscopic studies are an advantage in this exercise because many cells possess characteristic membranes and subcellular organelles that distinguish them from other cells which may look like them under the light microscope.

With man, even the skill possessed by the clinical pathologist has not prevented mistakes from time to time in the diagnosis of some common conditions. Thus the granulomatous pattern of Hodgkin's disease has been mistaken for tuberculosis and vice versa. I, myself, one time mistook the lesion of coccidioidomycosis for a clear cell carcinoma of the kidney, this after or perhaps because of too much reliance on the application of the periodic acid Schiff reaction for the demonstration of glycogen. My mistake is an example of where a fungal cell was mistaken for a cancer cell. Judging from some of the discussions at the workshop, this kind of mistake is a distinct possibility when trying to assign certain diseases of mollusks to a specific category—a parasitic infection or cancer.

Cellular immunity and other factors which control neoplasia, come into play with a variety of cancers of vertebrates. A common experience is with breast cancer in women, in whom the postoperative survival may vary from a few months to 25 or more years. Malignant melanoma may run a curious up-and-down clinical course. Patients with a kidney transplant, who are taking immunosuppressant drugs, may develop lymphoreticular type neoplasms of the brain, a nearly unheard of event in immunologically competent persons. Unexplained also is the vicious clinical course of the alveolar type rhabdomyosarcoma. It may appear suddenly in a localized part of a child's body and pursue a relentlessly fatal course in a brief period of 2 months. One must bear in mind that analogies may prove to exist between some of the debatable tumor-like lesions of man, the mouse, and mollusks. In children, in particular, considerable knowledge has accumulated on the morphology and behavior of pseudosarcoma, paradoxical fibrosarcoma and the like, lesions now considered not to be genuine neoplasms. With adults, too, certain proliferative fascial lesions present a histologic picture suggestive of a highly malignant tumor and yet these lesions do not pursue a malignant course.

With mice, the Moloney sarcoma virus induces a category of lesions classified as atypical granulomatous tumors with neoplastic-looking giant cells. These lesions tend to regress and disappear in immunologically competent mice but may evolve into genuine cancers in mice that are immunologically incompetent.

As the lantern slides, shown by the speakers at the workshop, were viewed by the other participants it became evident that some lesions diagnosed as cancer were considered by others to be non-neoplastic conditions. This is the same sort of evolutionary thinking that clinical and experimental pathologists experienced before they were able to separate neoplasms from certain non-neoplastic conditions in man and the mouse.

Development of further knowledge of the manifestations of neoplasia and neoplastic-like processes in the lower forms of animal life is also likely to throw light on some of the other mysteries connected with cancer that I have been talking about. While I cautioned earlier in my talk against too literal histologic comparisons among neoplasms of vertebrates this does not mean that important principles in oncology have not been established by comparative pathology studies. One need only recollect that the epochal discovery of the virus causation of cancer came from studies of leukemias and sarcomas of the domestic chicken. Temperature and altitude are critical factors in accounting for the geographic pathology of the Burkett tumor in the blacks of Africa. Moreover, there are striking differences in the frequencies of leukemia and glioma on the one hand and solid lymphoma and retinoblastoma on the other among black children living respectively in Washington, D.C. and Nigeria. With mollusks, the promotion and retardation of infections by viruses are known to be critically influenced by the temperature of the environment in which the animals are living. If viruses could be shown to induce cancer in mollusks then a model would be at hand to test one of the physical factors, namely, temperature, that influences certain cancers in human beings. Certain other neglected areas in the investigations of cancer in mollusks stand out. In the radiation experiments described at the workshop no mention was made about the effects of radiation on the induction of cancer in mollusks. This type of investigation should prove to be worthwhile as also would the exposure of mollusks to various known carcinogenic chemicals. The gills of fish exposed to aflatoxins have shown a curious affinity for the metastases of the hepatic cancers that arise from exposure to this carcinogen. Studies of phenomena like this strange pattern of metastasis might shed light on the spread of tumors in man.

[2]Farley, C. Austin. Oxford Laboratory, Middle Atlantic Coastal Fisheries Center, NMFS, NOAA, Oxford, MD 21654.

Comparative studies of cancer in primitive species have raised a critical problem centering about the evolutionary development of the thymic system of cellular immunity. To test the theory properly it is necessary to establish frequencies of tumors of invertebrate animals. This in turn depends upon the development of proper standards for the diagnosis of neoplasia in these lower forms. With its scope and dimensions and the prestige of the participants, the present workshop has gone a long way in these directions.

The accomplishments of the workshop will also have a bearing on a more practical problem. It is well known that the environment and habits of filter feeding mollusks place them in a position of exposure to carcinogenic agents. These undoubtedly account for some of the now known epizootics of neoplasms among mollusks. Scientists at the Oxford laboratory and other participants at this workshop are the pioneer leaders whose investigations of neoplasia in mollusks have placed an important tool in the hands of the environmental protectionists. Mollusks play an important role in the food chain. They are eaten by man and by bottom feeding fish. The fish are eaten by birds and they in turn by mammals. So mollusks, with their possible concentrated complement of carcinogens and poisons, make the full circle of the food chain. Moreover, the epizootics alert the environmentalists about the possible exposure of man to carcinogens in bays and estuaries and in the streams and rivers that flow into them. This can be an important clue leading to the identification of the source of the pollution which may be adversely affecting exposed people in the neighborhood of the source, as well as those living downstream who obtain their domestic water supply from the effluent.

From these remarks the value of this workshop is obvious.

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MFR PAPER 1216

The Workshop on Molluscan Pathology: Closing Comments

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During the past several days, a number of very interesting lesions have been presented, discussed, and studied firsthand, by the participants from microscopic slides of material submitted by the various speakers. It is clear that much remains to be learned before we can make intelligent judgements about the nature of the various proliferative lesions of mollusks. A case in point is our knowledge concerning the kinetics and differentiation of hematopoietic cells in oysters, and I suspect, other molluscan species which appear quite rudimentary. Studies in this area must be expanded if we are ever to understand and accurately classify the various proliferative conditions of this system.

Since it has been amply demonstrated that lesions suspiciously resembling neoplasms occur in bivalves in nature, it would now seem a logical step to begin carefully controlled laboratory experiments in which the effects of known and suspected carcinogens are studied, not only with respect to biological and pathological phenomena, but biochemical and metabolic ones as well. It still remains to be established whether marine invertebrates possess the mixed function oxidases found in higher animals, enzymes necessary for the metabolic in vivo conversion of a variety of chemicals including hydrocarbons to carcinogenic compounds. To date, evidence implicating aquatic pollutants in the genesis of these lesions has been purely circumstantial. Guilt by association is not a convincing case. A direct cause and effect must be demonstrated between a substance such as a hydrocarbon present in fuel oil, for example, and the ultimate development of a "tumor". The autonomous nature of such lesions should be established by transplantability in genetically controlled molluscan hosts. Finally, it seems that very little is known about the natural history of proliferative lesions in mollusks due largely to the fact that these mollusks are encountered sporadically in the field and are either dead or dying, offering little or no opportunity for study. Physiological studies of affected animals must be undertaken under carefully controlled laboratory conditions if a better understanding of how such lesions interfere with normal function is to be forthcoming. It is hoped this conference will serve to stimulate further research in this fascinating and important area of invertebrate pathobiology. Thank you all, speakers and participants alike, for a successful conference.

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NOAA's Nautical Charts and Weather Information Essential for Boating Safety

Most motorboat operators involved in collisions or grounding accidents are not newcomers to the water, according to the Commerce Department's National Oceanic and Atmospheric Administration.

A profile of the accident-prone boater, drawn from the more than 4,500 collision or groundings reported to the U.S. Coast Guard last year, shows that the operator was between 26 and 50 years old with over 500 hours operating experience. In more than half of the accidents reported, he or she was operating a fiberglass open motorboat between 16 and 26 feet in length with an outboard engine of over 75 hp.

NOAA, whose nautical charts and weather services are used by boaters year-round has added a new service—the Loran-C lines of position for long range navigation—to over 100 nautical charts since the program was launched a year ago. The Loran-C chart program is one of a number of programs taken to advance boating safety and to assist recreational boaters, fishermen, and commercial shipping by NOAA's National Ocean Survey, National Weather Service, and the National Marine Fisheries Service and the Commerce Department's National Bureau of Standards.

NOAA's National Ocean Survey has updated and printed new editions of approximately 550 nautical charts. In addition, four new charts, one reconstructed chart and one new training chart were published, and the limits of two charts were extended.

To help prevent collisions, traffic separation schemes implemented by the U.S. Coast Guard to separate inbound and outbound traffic were added to the following charts: Strait of Juan de Fuca, Puget Sound and the Strait of Georgia, Washington; Prince William Sound, Alaska; and the Delaware-Cape Henlopen approach to Delaware Bay was realigned. In addition, safety fairways in the approaches to Corpus Christi, Texas; Pensacola Bay, St. Andrews Bay, Port St. Joe, Charlotte Harbor and Tampa Bay, Florida were revised.

In a further effort to promote boating safety, NOAA's marine centers in Norfolk, Va., and Seattle, Wash., are sponsoring Cooperative Charting and Chart Updating Seminars for leaders of the U.S. Power Squadrons and U.S. Coast Guard Auxiliary to equip them better for volunteer investigations in support of the nationwide chart correction programs.

A new training manual on Cooperative Charting has been prepared by the U.S. Power Squadrons with technical advice from the National Ocean Survey. It is designed to help volunteer observers of the U.S.P.S. in chart deficiency survey activities and to improve volunteer chart correction work, thus fostering safer navigation through up-to-date nautical charts and related publications.

A major role in NOAA's program to increase boating safety is carried out by the National Weather Service. This NOAA agency prepares forecasts for shore areas of the U.S. every six hours, more often when conditions change rapidly. Forecasts cover specific coastal areas, such as Block Island, R.I., to Manasquan, N.J. When strong winds or hazardous seas are anticipated, these forecasts include statements of the degree of hazard and the areas where warning signals will be displayed. Similar forecasts and warnings are issued for the Great Lakes and many inland lakes, reservoirs and waterways.

Boaters also receive pertinent weather information over commercial radio and TV. Most stations in coastal regions make a special effort to answer the needs of boaters and shipping in their weather forecasts.

In a growing number of shore areas, weather information can now be received through VHF-FM radio stations operated by the National Weather Service. The more than 60 stations along the coastal areas are on the air continuously, repeating taped weather messages every four to six minutes, 24 hours a day, seven days a week. Tapes are updated periodically, usually every two to three hours, and revised also to meet fast-changing weather. Special receivers or tuners are required since the weather forecasts are made on 162.40, 162.475, and 162.55 MHz.

As a supplement to NOAA Weather Radio, the U.S. Coast Guard broadcasts the National Weather Service's forecast four times a day from stations strategically positioned on the nation's coast line.

In addition to its long-range weather broadcasts, the Coast Guard has 54 Coast Guard stations broadcasting with short-range VHF-FM radio transmitters. Other information available to the mariner is from the National Bureau of Standards time signals which are broadcast world wide over their stations WWV (Ft. Collins, Colo.) and WWVH (Kauai, Hawaii). Imposed on the time signal for certain portions of each hour is storm information for deep water vessels.

The National Weather Service has also established a more widespread distribution of its "Marine Weather Services Charts," which list weather information for various coastal areas and the Great Lakes. These charts are available from the National Ocean Survey's nautical chart sales agents.

The National Marine Fisheries Service also has safety placards available again to commercial fishing vessels on how to deal with emergency problems at sea. The placards will be suitable for posting aboard the vessels.

ALASKA OCS Ecological Program Adds Chukchi Sea, Norton Sound Areas

The National Oceanic and Atmospheric Administration's Outer Continental Shelf Environmental Assessment Program has been expanded to cover prospective oil-lease areas off Alaska's northwest coast, the Commerce Department agency has announced.

The $8 million, multi-year study of life forms and environment in Alaska's Norton Sound and Chukchi Sea is part of a continuing investigation conducted by NOAA's Environmental Research Laboratories for the Interior Department's Bureau of Land Management. It extends work already underway in the Bering Sea, the Gulf of Alaska, and the Beaufort Sea, which links Alaska to the Arctic Ocean.

Emphasis in the program will be on conducting reconnaissance work to identify the crucial biological and physical elements of the Alaska marine ecosystem. These findings will be used to establish scientific baselines for the regions, against which petroleum-connected impacts can be detected, assessed, and monitored.

Design of the Chukchi Sea-Norton Sound study has been guided by more than a year and a half of such investigations in other outer continental shelf areas off Alaska, according to Rudolf J. Engelmann, who directs the program for NOAA.

"Given the time constraints of oil leasing and development schedules," he explains, "we've had to narrow our investigation. Our first year and a half up here taught us that we're not going to obtain a full statistical description and explanation of the environment in a reasonable time, at tolerable cost. The natural variability of the ecosystem is too great, the species are too mobile, and the environment is too hostile to accomplish that.

"Instead, we are doing reconnaissance to identify key species and habitats and then placing high priority on determining how they will be affected by petroleum development. We are also doing our best to incorporate those items needed for assessment and environmental protection.

"This type of selective investigation is cutting the time it takes to develop the data, models, and methods needed for assessment of environmental impact."

As in the other Alaskan study areas, much of the Chukchi Sea-Norton Sound research will be performed by scientists in other government agencies, universities, and private industry, working as NOAA subcontractors.

Two-Man Research Sub Gathers Marine Data

Studies ranging from the geology of potential offshore oil drilling sites to the ecology of fish and shellfish were carried out from a two-man research submarine operating off the northeast coast from mid-June to 1 August, the Commerce Department reports.

Nekton Gamma, the tiny underwater workhorse of General Oceanographics, Inc.[1], carried scientists to study sites deep in the ocean under contract with the Commerce Department's National Oceanic and Atmospheric Administration (NOAA). The cooperative scientific-exploratory program is carried out by the U.S. Geological Survey, the U.S. Army Corps of Engineers, and NOAA's Manned Undersea Science and Technology office.

Richard Cooper of the Northeast Fisheries Center in Woods Hole, Mass., part of NOAA's National Marine Fisheries Service, used the submersible in July to conduct ecological studies of areas in Stellwagen Bank and the Gulf of Maine. He investigated bottomfish and shellfish behavior and distribution, as well as ecological factors affecting survival of the animals in their egg and larval states.

David Folger of the U.S. Geological Survey, also from Woods Hole, made bottom geological observations in expected oil-drilling areas of Georges Bank and the Baltimore Canyon trough 1-15 July as part of the program.

Biological studies of ocean dumping sites were carried out during mid-July by Gilbert Chase of the U.S. Army Corps of Engineers, Waltham, Mass. His work involved dives on the Brenton Reef ocean dumping site and the Rhode Island sound shelf area.

Finally, George Freeland of NOAA's Atlantic Oceanographic and Meteorological Laboratories, Miami, Fla., gathered geological and biological information on the seafloor of the New York Bight during dives from 21 July to 1 August. This work was part of Freeland's ongoing research in the area in connection with the Marine Ecosystem Analysis program undertaken by NOAA's Environmental Research Laboratories.

Nekton Gamma can take a pilot and scientist to depths of 1,000 feet for bottom sampling and photographic and direct observation of conditions on the continental shelf and slope that affect or are caused both by natural events and man's activities.

West Coast Satellite Fish Data Expanded

An innovative method of using satellite-provided information to help tuna and salmon fishermen find more productive fishing grounds off California's coast has been expanded this season according to the National Oceanic and Atmospheric Administration (NOAA).

The method, proven of worth in a pilot project last year, involves advising commercial fishermen where "upwelling" occurs in coastal Pacific waters. A NOAA polar-orbiting satellite, equipped with infrared sensors, can identify the cold, nutrient-rich waters associated with upwelling, and provides a picture showing their locations. Salmon and tuna favor these upwelled waters.

Satellite coverage has now been expanded to include the area from the Strait of Juan de Fuca (between Canada and Washington) to Point Concepcion, Calif., and information from the satellite is now more readily available to fishermen through use of an automatic telecopier that transmits thermal front data to the dockside more quickly.

The service to fishermen is provided jointly by NOAA's National Environmental Satellite Service and the NOAA-sponsored Sea Grant program at Humboldt State University, Arcata, Calif. NOAA is an agency of the Department of Commerce.

The NOAA satellite, equipped with both visual and infrared sensors, passes over the coastal area twice daily, relaying environmental data including sea surface temperatures back to earth.

This information is converted into images in various shades of gray, each shade representing a particular interval of temperature. As a result, says NESS Oceanographer Larry Breaker, thermal fronts where cold, upwelled water meets the warmer offshore surface water show up clearly as gray-shade boundaries.

The locations of thermal fronts are transferred to navigation charts, copies of which are then furnished to fisher-

[1]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

men at various ports. This chart can also be transmitted to anyone equipped with a telephone and a telecopier. The coordinates of this fishing broadcast by the National Marine Fisheries Service and Western Fishboat Owners Association in San Diego is also transmitted on single sideband radio. The technique may have applications for fishermen in many parts of the world.

Fishermen and oceanographers have known for years that as strong winds from the north and northwest blow along California's coast, coastal surface water is moved offshore and is replaced through upwelling.

Bottom water, according to Fred Jurick, a Sea Grant Marine Advisory agent at Humboldt State, is rich in nutrients which, when they come to the surface, cause huge blooms of plankton, the primary food source for marine life. This attracts bait fish, which in turn attract tuna and salmon.

In recent years, efforts to plot the location of upwelling have included taking infrared temperature readings from aircraft. Such attempts often were successful, but had drawbacks such as weather restrictions on operations, limited aircraft range and high cost.

The NESS-Sea Grant program is only one of several projects utilizing NOAA satellites and aimed at assisting fishermen. A second effort shows promise of pinpointing concentrations of algae in water by satellite infrared identification of the chlorophyll in the marine plants. Many kinds of fish tend to congregate in algae-rich waters. The chlorophyll project uses infrared sensors aboard NOAA satellites which are in geostationary orbit above the equator.

Environmental Monitor Launched for NOAA

An environmental monitoring satellite, providing pictures and other data of value to such varied interests as weather forecasters, fishermen, aviators, oceanographers, and hydrologists, was launched at Lompoc, Calif. on 29 July for the National Oceanic and Atmospheric Administration (NOAA), the Commerce Department reports.

The spacecraft, put into polar orbit for NOAA by the National Aeronautics and Space Administration from its site at the Western Test Range, is known as NOAA-5, representing the fifth satellite of this type to be operated by the Commerce Department agency.

Two of the previous four NOAA series of polar-orbiting spacecraft still provide limited information, but after 2½ years and 1½ years in orbit, respectively, are deteriorating from the harsh environmental conditions 900 miles (1,440 kilometers) in space.

The orbit of the new spacecraft lets it scan the entire globe twice every 24 hours. It is moving westward on each orbit so that at any given location on the Equator the satellite crosses the Equator headed southbound at 8:30 a.m., local time, and northbound at 8:30 p.m.

The launch of NOAA-5 maintains an operational satellite system begun some 10 years ago that today provides regular global observations of cloud cover, snow and ice, and the sea surface for an ever-increasing variety of uses.

Initially, polar-orbiting spacecraft were used to provide meteorologists with additional information from which to make weather forecasts. But throughout the years other uses of the visual and infrared images have been found by NOAA's National Environmental Satellite Service.

Satellite pictures have helped in the control of locusts in Africa, have provided information on ice in the Great Lakes and around Alaska, and have been at least partly responsible for saving thousands of lives by early detection and tracking of hurricanes and tropical storms.

More recently, data from the polar-orbiting satellites (NOAA also operates three geostationary satellites) have been put to use by both the U.S. Coast Guard and the Civil Air Patrol in their respective search and rescue missions; by tuna and salmon fishermen off the West Coast, helping them find more productive fishing grounds; and by planners who use imagery to help predict how much water from snow melt in the mountains and higher elevations will flow into rivers, streams and reservoirs.

Additionally, more than 120 nations in all parts of the world receive imagery from the NOAA series of satellites. Government agencies, academic institutions, scientific groups and even amateurs use the data for many purposes in what is considered one of the most successful demonstrations of peaceful uses of space technology.

The new spacecraft, known at ITOS-H until it achieved orbit, is a box measuring 40 × 40 × 49 inches, and has three wing-like panels to which solar power cells are attached. Four major sensing systems are included as part of the main body of the spacecraft, and each has a back-up system for more reliable service and longer lifetime.

The spacecraft manufacturer, RCA, has had 24 polar-orbiting satellites of this general type successfully launched since 1960, a number of them research and development spacecraft operated by NASA. During that time, the satellites have returned some three million views of the world's weather to earth, and since 1966 no major storm has gone unobserved by them.

The sensing systems carried into space on NOAA-5 are:

1) A Scanning Radiometer System to gather data in both visible and infrared channels, scanning a swath about 2,000 nautical miles wide beneath the spacecraft's path. The system provides both stored picture coverage for transmission upon demand, and direct transmission of pictures to receiving stations within range. The visible channel resolution is two nautical miles (features two miles by two miles in size or larger can be seen on the imagery) while the infrared resolution is four miles.

2) A Very High Resolution Radiometer System to obtain higher resolution, in both visible and infrared channels, than does the scanning radiometer. VHRR images can show features one-half mile in size.

3) A Vertical Temperature Profile Radiometer System to measure infrared energy radiated at six levels of the atmosphere and at the earth's surface or cloud tops for measuring vertical temperature distribution, and to provide information on the total moisture content of the atmospheric column observed.

4) A Solar Proton Monitoring System to detect the arrival of energetic solar protons in the vicinity of the earth, for use in issuing warnings of solar storms.

Economic Impact of Marine Angling Eyed

The effects of marine recreational fishing upon this country's economy will be studied under a National Oceanic and Atmospheric Administration (NOAA) contract issued last summer.

The contractor, Centaur Management Consultants, Inc., of Washington, D.C., will gather information on the value of goods and services generated by the sport.

As saltwater anglers use fishing equipment, boat rentals, motors, camping equipment, food, and lodging, the survey will identify and evaluate the commerce created by the sale of these items.

The $70,329 contract, awarded by NOAA's National Marine Fisheries Service, will divide the economic impact into four regional areas: Northeast, southeast, northwest, and southwest.

Sources of information will include sampling surveys of supplies of goods and services from origin to point of sale. Other data may be supplied by state and public agencies, by private organizations, and by trade associations.

Among Federal agencies, the NMFS is responsible for the development of conservation and management policies for marine fisheries resources for both commercial and recreational purposes. Over the years, a considerable body of knowledge has accumulated on the economics of commercial fisheries, but relatively little on recreational fishing.

The end product of the study will be a comprehensive document of the socio-economic scope and value of marine recreational fishing in the United States. The study, to be completed in the spring of 1977, will assist the NMFS, state agencies, and regional management councils in evaluating the relative merits of recreational and commercial fishing in the management of marine resources and in administering the Fishery Conservation and Management Act of 1976.

Foreign Fishery Developments

Egyptian Fishery Developments Noted

Admiral Emad El Din Madkour, Managing Director of the Egyptian High-Seas Fishing Company (a government-owned corporation), traveled to Aden in early April 1976 to conclude an agreement for a proposed joint Egypt/People's Democratic Republic of Yemen Fisheries Company. However, the Admiral has indicated that all financial problems associated with the formation of this new joint venture have not been solved. The proposal requires an initial investment of US$5 million which will be paid by the two partners on signing the agreement; the remaining $20 million is to be paid in equal amounts by both partners at the rate of US$5 million annually within the next 4 years. The company will own and operate one large refrigerated fish carrier and a fleet of eight fishing vessels. The company plans to fish in the southern Red Sea, the Persian Gulf, and the Indian Ocean. Admiral Madkour said that, based on his company's past experience, the initial US$5 million will not be sufficient. The Egyptian High-Seas Fishing Company was to have had a capitalization of US$14 million in 1964 to acquire 21 fishing and fish-transporting vessels. The Egyptian Government reduced the capitalization to $3 million. Even with sizeable loans, the Company was able to acquire only five used vessels, two with a capacity of 1,700 GRT and three having 650 GRT. Admiral Madkour is now seeking US$4.5 million (including a request to the US Agency for International Development) to refit and modernize these vessels.

The Egyptian High-Seas Fishing Company does not have any vessels fishing in the proposed fishing area of the southern Red Sea and northern Indian Ocean where the Admiral has indicated that lobster and shrimp are plentiful. Despite occasional calls by Egyptian Government planners for greatly increasing the supply of fish to help meet Egyptian protein needs, Admiral Madkour seems to get very little help in his efforts. The present annual catch of the Company's fleet is only 15,000 metric tons. By modernizing and expanding the fleet to the 21 vessels originally planned, Admiral Madkour says he could increase the company's catch to 100,000 metric tons per year. (Source: U.S. Consulate General, Alexandria.)

JOINT VENTURE WITH GDR

Late in 1975, Egypt announced the formation of a joint fishing company to exploit the fishing resources of Lake Nasser. With the technical assistance of experts from the German Democratic Republic (GDR — East Germany), the company intends to operate a fleet of 16 fishing vessels in the Aswan Dam's Lake Nasser, and construct three cold-storage plants capable of holding 1,000 metric tons of catch each. The company will also acquire a fleet of refrigerated trucks to transport the catch to population centers. (Source: U.S. Embassy, Cairo.)

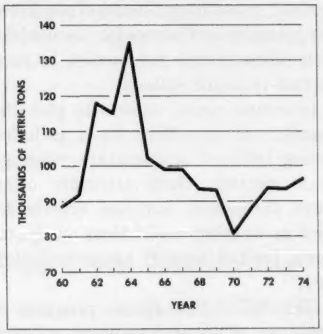

Figure 1.—Egyptian fisheries catch, 1960-74.

JOINT VENTURE WITH NORWAY

Negotiations are about to be concluded with the Norwegian firm Hareide International Group to develop fishing resources in Lake Nasser. Production is expected to double within 1 year. Hareide International will also submit a 5-year development plan to the Egyptian Government to expand Egyptian fishing in the Mediterranean and the Red Sea. (Source: *La Pêche Maritime*.)

DEVELOPMENTS IN LAKE MARYUT

Pro-fisheries forces seem to have won a low-key struggle over the future of Lake Maryut, a sizeable expanse of

shallow fresh water south of Alexandria. According to *Progres Egyptien*, the Governor of Alexandria, Abdel Tawab Hodeib, has ordered a halt to any further draining or landfilling of Lake Maryut. A strong lobby of agriculturists and real estate promoters had been urging more extensive drainage of Maryut to provide both badly needed farmland and additional building sites. The entire Smouha quarter of the city was formerly part of Lake Maryut, including the extensive Nouzha area where Alexandria's municipal airport was built. As the airport is under military control, fishers have not been allowed to fish the Nouzha grounds of the lake.

Governor Hodeib's special Committee of Inquiry for Food Resources has recommended that steps be taken to stop industrial pollution of Lake Maryut (the western reaches of the lake lie in an industrial area). The Committee also recommended that the present water levels be maintained and the lake restocked. The Committee further recommended that "competent authorities" (i.e., the Ministry of Defense) be asked to reopen the Nouzha grounds so the resources that have not been fished in recent years can be utilized. (Source: U.S. Consulate General, Alexandria.)

FISHERIES CATCH

The total Egyptian fisheries catch in 1974 was 96,200 metric tons, an increase of three percent over the 93,500 metric tons caught in 1973 (Fig. 1). A record 135,000 metric tons was caught in 1964. Since 1966, catches have been below 100,000 metric tons, reaching a low of 80,900 metric tons in 1970. While more recent catches have recovered somewhat from the 1970 low, the 1974 catch is still 29 percent below the record catch of 1964.

Japanese Fish Farm Will Use Nuclear Power Plant Hot Water Discharges

The Prefectural Government of Fukui, Japan, has announced plans to begin construction of a $600,000 fish farm this year. The farm will be built in Urazoko Bay, and will use the hot water discharges from a nuclear power plant. The farm will consist of two areas of the bay enclosed by netting located about 500 meters from the power plant.

The farm plans to raise young "ayu" (sweetfish), red sea bream, and yellowtail. As yellowtail cannot winter in the 3-4°C waters along the Japan Sea coast, this project aims at keeping the water temperature in the fish enclosures at 10°C by using the nuclear power plant's hot water wastes. Initial experimentation began this autumn when the enclosures were stocked with 20,000 young yellowtail and 15,000 red sea bream.

In addition, the prefectural Government will stock a freshwater research pond with 160,000 young "ayu," a popular domestic freshwater fish. This will be the first attempt at "ayu" farming in Japan.

According to the NMFS Office of International Fisheries, in a similar project last winter, a Fukui fishery cooperative succeeded in wintering juvenile yellowtail in a small enclosed area supplied with hot water discharges from a nuclear power plant. If the larger scale project is successful, similar farms may eventually develop around the nine nuclear power plants to be built in the Wakasa Bay area, Fukui, Japan. (Source: *The Japan Times*.)

Russia Orders Polish Fish Factory Vessels

The Soviet Union has placed a new and valuable order in Poland for the construction of 11 fish factory baseships of the B-670 class, according to the NMFS Office of International Fisheries. The contract, worth more than US$250 million, is the most valuable single order in the history of the Polish shipbuilding industry.

The B-670 contract was first discussed publicly during Inrybprom-75, the international fisheries exhibition which was held in August 1975 in Leningrad. However, it was not until 9 December 1975 that a contract was signed in Moscow for the purchase of six B-670-class vessels. Later, on 13 January 1976, it was announced that the Soviets had ordered a total of 11 fish factory baseships of the B-670 class. Delivery of the vessels will take place from 1978 through 1980.

Little is known thus far about the basic specifications for the new B-670 class, which was designed to supersede the older B-69 series. According to Polish sources, the vessels will be much more sophisticated than the B-69 class. They will be 178.3 meters long and 11,500 tons deadweight, and be able to attain a speed of 15 knots. The processing facilities aboard will consist of eight different production lines. The vessel's primary processing function will be to produce 220,000 cans of sardines, saury, mackerel, and other pelagic fish in 24 hours. The freezing line will be capable of processing 100 tons of fresh fish in 24 hours, the salting line 150 tons in barrels and 20 tons in cans during the same period. Production potential also includes 50 tons of salted fish in 50-liter barrels, 3 tons of roe, 4.5 tons of cod liver oil, and 100 tons of fish meal each day. By comparison, the B-69-class factory baseships only have the capacity to process 90 tons of fish each day, fillet 80 tons of cod, and produce 165 tons of salted herring in barrels per day.

The fish factory baseship *Professor Baranov*, the first vessel of the B-69 class, was delivered to the Soviet Union in 1967 by the Polish shipyard Stocznia Gdanska. The Soviet Ministry of Fisheries ordered a total of 35 of these vessels from Poland; the last was delivered to the Soviet fishing fleet at the end of 1975.

The B-69-class of fish factory baseships (also known as the *Professor Baranov* class after the prototype vessel) was designed for use by the Soviet high-seas fishing fleet in the Atlantic. Vessels of this class are equipped with elaborate crew quarters and processing equipment capable of producing finished as well as semi-finished products. The basic specifications of this class are as follows:

Length (m)	164.0
Width (m)	21.3
Draft (m)	8.08
Deadweight Tons	10,000
Gross Register Tons	13,571
Horsepower	7,200
Speed (knots, max.)	15.7
Crew	254
Endurance (days)	75

According to the NMFS Office of International Fisheries, most coastal nations, either unilaterally or through the U.N. Law of the Sea Conference, are expected to extend their fishing

limits to 200-mile exclusive economic zones in the near future. It therefore seems likely that countries (such as the Soviet Union) which harvest the majority of their fisheries catch off foreign shores will be forced to cut back fishing operations in those areas. However, the Polish shipbuilding industry, which has sold fishing vessels to the Soviet Ministry of fisheries since the 1950's, requires a constant flow of new orders to remain profitable. Polish sources have indicated that this new $250 million order, coupled with orders for new "supertrawlers" and tuna purse seiners, will provide future investment funds for the Polish shipbuilding industry. According to Jerzy Hinc, Export Director of the Centromor Polish Vessel Import/Export Company, the orders will not only earn foreign currency, but also "create prospects for our (Polish) shipbuilding industry till the end of the century."

Publications

Foreign Fisheries Articles Translated

Listed below are abstracts of articles on foreign fisheries recently translated by the Language Services Division, Office of International Fisheries, National Marine Fisheries Service, NOAA. Copies of the full translations are available from the Language Services Division, F43, National Marine Fisheries Service, NOAA, Washington, DC 20235.

"Whither 'Ethnic Purity'?" Under this title, an article in *Suisan Shimbun*, No. 3705, 17 March 1976, reports that an unidentified Japanese trading company is arranging for the chartering of Korean shrimp trawlers in Surinam. The Surinam-Japanese company SUJAFI is currently operating 50 shrimping vessels. Five of these vessels are Korean trawlers chartered by SUJAFI through the unnamed Japanese trading firm. SUJAFI reportedly wishes to charter 15 more Korean vessels. More than 80 Korean fishing vessels are in operation on the shrimping grounds off Surinam. The same trading firm arranged for 39 of these 80 vessels to fish there. The writer of the article finds these activities of the trading firm difficult to reconcile with the diminishing role confronting Japanese fishing companies in Surinam. (Translator's comments: The Japanese fishing industry is very sensitive about the Korean inroads into distant water fisheries. The frank tone of the article is interesting, because *Suisan Shuho* is an official publication of the Japan Fisheries Association. The Association president will be the chief negotiator in the forthcoming U.S.-Japan fisheries bilateral talks.)

"Japanese Albacore Prices Soar." According to an article in *Asahi Shimbun*, No. 32501, 19 June 1976, wholesale albacore prices at the tuna-landing port of Yaizu are soaring this season. The average price as of the end of May was 380 yen/kg (US$1.27/kg), which was about twice as much as the average price for the same period last year. This sudden price increase has been attributed to frantic albacore buying at Yaizu by several American canners. The article states that the race to obtain albacore for canning has been spurred by the ban on tuna fishing in the 200-mile economic zone just imposed by Mexico.

"A 4.5 Million Metric Ton Decrease in Fish Catches." According to an article in *Suisan Shimbun*, No. 3710, 31 March 1976, the annual Japanese catches would decrease by about 4.5 million metric tons if all coastal nations of the world established 200-mile economic zones. Out of this total, the eleven largest fishing companies of Japan would suffer about a 2.4 million metric ton decrease, while the small to medium-sized fishing companies would suffer a 2.1 million metric ton decrease. The latter figure is broken down in the following table:

Offshore trawling (single vessel)	463,900
Offshore trawling (pair)	2,400
Northern Seas (East and West)	927,700
Shrimp trawling (South America)	194,400
Large and medium-size purse seining (single)	145,600
(pair)	—
Salmon drift netting	11,700
Salmon longlining	1,400
Salmon mothership fleet	19,100
Crab (northern, tanner)	3,500
Gill netting	22,600
Crab mothership	16,400
Northern Seas longlining and gill netting	29,800
Tuna (incl. skipjack)	266,700
Total	2,108,800

Recent reductions in the number of Surinam-based Japanese fishing vessels.

Companies	From	To	Companies	From	To
Nisshin Co.	28	23	Zen Sake Ren	6	6 (To Nisshin)
Shinyo	18	13	Guyana Suisan	5	(Moved to other bases)
Hokoky	8	3	Nippon Enyo	8	
Kagawa	8	3	Nippon Kyodo	8	(To Brazil Taiyo)
Nambei Ebi	(8)	(Bankrupted)	Hakodate	8	8
Seiwa	5	(all Korean crew)	Yutaka	13	13
			Total	123	69

Fishery Hygiene Booklet Noted

"Fish and Shellfish Hygiene," published by the Food and Agriculture Organization (FAO) of the United Nations, is the report of a World Health Organization (WHO) Expert Committee on Fish and Shellfish Hygiene convened cooperatively with FAO in September 1973.

The 62-page booklet briefly reviews principal human diseases associated with fish and shellfish and current public health problems related to their production, processing, and distribution. Diseases caused by living organisms are better covered than those caused by biotoxins or chemical pollutants. Some environmental factors are briefly discussed, as are scientific and administrative aspects of hygiene problems. Appendices include characteristics of principal parasitic fish- and shellfish-born diseases in man and a selected bibliography. The volume is available from Unipub, Box 433, Murray Hill Station, New York, NY 10016 at $2.50 per copy.

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